

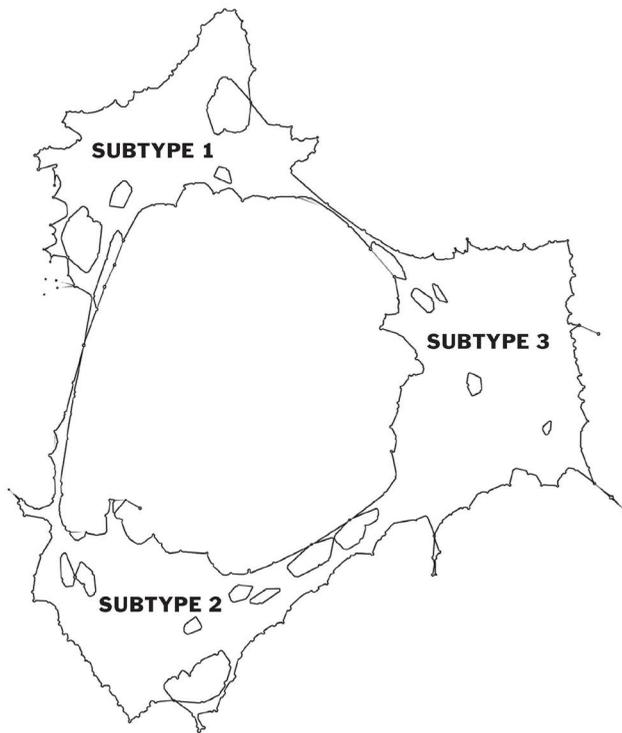
NIDDK

Recent Advances &
Emerging Opportunities

February 2017



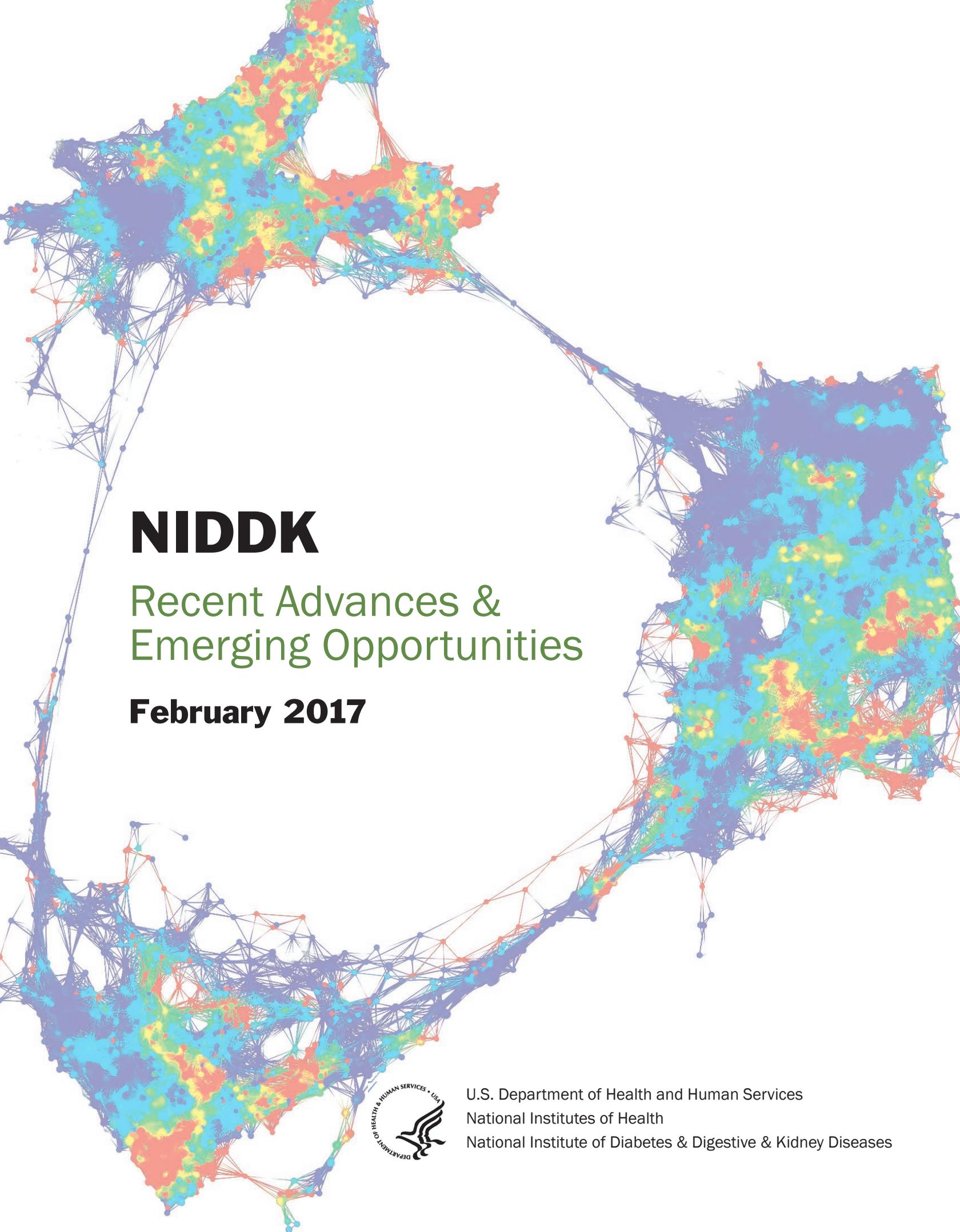
U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases



Cover caption: NIDDK-supported researchers analyzed data from the electronic health records of more than 11,000 volunteers, many with type 2 diabetes. This led to the discovery of three distinct subtypes of type 2 diabetes, as represented on the cover (and in the outline above) by three separate clusters of patients (females in red/orange/yellow, and males in blue/green) that emerged when the people with type 2 diabetes were grouped based on similarities in clinical features. The subtypes showed differences in risk for specific diabetes complications, such as heart disease, and comorbidities, such as cancer. This type of approach could provide a more precise understanding of type 2 diabetes, lead to personalized therapies for people with different disease subtypes, and be applied to other diseases within the NIDDK's mission. It also gives a preview of the type of knowledge that could stem from the National Institutes of Health's All of UsSM Research Program, which will be collecting extensive data contributed by over 1 million volunteers to extend the benefits of precision medicine to many diseases.

The NIDDK supports research toward precision medicine, which aims to move away from a "one size fits all" approach so that disease prevention and treatment decisions will instead be personalized and based on individual variability in genes, environment, and lifestyle. Precision medicine has the potential to allow health care that predicts more accurately which prevention and treatment strategies will work in specific people.

Image courtesy of Dr. Joel Dudley, Icahn School of Medicine at Mount Sinai, New York. From: Li L, Cheng WY, Glicksberg BS, Gottesman O, Tamler R, Chen R, Bottinger EP, and Dudley JT. Identification of type 2 diabetes subgroups through topological analysis of patient similarity. *Sci Transl Med.* 7: 311ra174, 2015. Reprinted with permission from AAAS.



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

TABLE OF CONTENTS

MESSAGE FROM THE DIRECTOR

CROSS-CUTTING SCIENCE 1

Understanding Mitochondria's
Response to Stress 1

*Stressed to the Breaking Point—
How a Cell's Mitochondria Respond
to Stress To Maintain Health* 1

Insights into Wound Repair 2

*The Ties That Bind in Tissue
Scaffold Assembly* 2

Dr. Gary Felsenfeld Receives Horwitz Prize:
NIDDK Intramural Research Program Scientist
Pioneered the Field of Epigenetics 3

NIDDK-supported Scientists Receive
Presidential Award 4

Precision Medicine—Moving Away from
“One Size Fits All” Medical Treatments 5

Molecular Transducers of Physical Activity 6

Workshop on Opportunities for
Simulation Research in Gastrointestinal
and Urologic Care 7

DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES 9

Type 1 Diabetes—Health Benefits
of Good Glucose Management 11

*Intensive Blood Glucose Management for Those
with Type 1 Diabetes Preserves Heart Health
and Reduces Risk of Early Mortality* 11

Beta Cells and Diabetes 12

*Making Beta Cells from People with
Type 1 Diabetes* 12

*New Biomaterial Protects Transplanted
Insulin-producing Beta Cells from the
Immune System* 13

*Newly Discovered Proteins in Beta Cells Are
Targets for Autoimmune Attacks Implicated
in Type 1 Diabetes* 13

*Not All Beta Cells Are Alike—Discovery
Helps Explain Altered Insulin Secretion
in Type 2 Diabetes* 14

The Human Islet Research Network: Toward
Innovative Strategies for the Treatment and
Prevention of Type 1 Diabetes 16

Combating Type 2 Diabetes in Youth 17

*TODAY Study Helps Predict Whose Blood
Glucose Will Rise Tomorrow* 17

Screening for Prediabetes 18

*Improving on Methods for Diagnosing
Prediabetes in Africans* 18

Genetics of Type 2 Diabetes 18

*Genetic Trait in Pima Indians Linked to
Increased Birth Weight and Elevated
Risk for Type 2 Diabetes* 18

<i>Variation in a Glucose Transporter Affects Response to the Type 2 Diabetes Drug, Metformin</i>	19	Scientific Presentation: Dr. Alan R. Saltiel— Out of Balance: Obesity, Inflammation, and Diabetes.....	31
Diabetes Portal Adds Data, More Powerful Search Tools: Detailed Content Now Available to Everyone	20	Patient Profile: Sisters Participate in Life-changing Clinical Trials—Testing Artificial Pancreas Technology for Managing Type 1 Diabetes.....	34
Molecular Underpinnings of Glucose Control and Insulin Resistance.....	21	Patient Profile: A Life in Public Health and Service to the Community Continues with Participation in the GRADE Study for Type 2 Diabetes	39
<i>A Molecular Signal That May Lead to Insulin Resistance and Type 2 Diabetes</i>	21		
<i>Newly Identified Molecule Modulates Glucose Release by the Liver</i>	21		
Metabolic Regulators of Health and Disease.....	22	OBESITY	45
<i>Brain Cells That Control the Body’s Response to Heat</i>	22	The Brain’s Regulation of Appetite and Digestion.....	45
<i>Identification of Exercise Molecule That Promotes Physical Endurance</i>	23	<i>New Insights into How the Brain Handles Hunger</i>	45
Cystic Fibrosis Research	23	<i>The Inner Workings of a Brain Cell That Drives Eating and Weight Gain</i>	46
<i>Understanding Differences Between Mouse and Human Cystic Fibrosis Suggests a Potential Way To Reduce Infections</i>	23	<i>A Nutrient Sensor in Brain Cells Regulates Feeding</i>	47
Screening for Lysosomal Storage Disease	24	<i>How the Brain Knows When the Stomach Has Stretched and There Is Food To Digest</i>	47
<i>Improving Newborn Screening for Lysosomal Storage Diseases</i>	24	New Directions in Obesity Treatments	48
Story of Discovery: Islet Transplantation— A Promising Treatment for Difficult-to-treat Type 1 Diabetes	26	<i>Brain Stimulation with Electric Current Leads to Changes in Food Consumption and Body Weight in a Preliminary Study of Adults with Obesity</i>	48
		<i>Naturally Occurring Compound Shows Potential as a Treatment for Obesity and Diabetes</i>	48

<i>Custom-made Fat Tissue That Burns Calories</i>	49	<i>Understanding How Crohn’s Disease Treatments Affect Children’s Gut Microbiome</i>	62
Insights into Weight Loss Maintenance	50	Genetic Underpinnings of Inflammatory Bowel Disease	63
<i>Research Reveals Persistent Metabolic Slowing as Potential Catch-22 in Maintaining Significant Weight Loss</i>	50	<i>Delving into Genetics To Gain a More Personalized View of Inflammatory Bowel Disease</i>	63
Discoveries Provide Insights on Eating Behavior	50	<i>Exploring the Genes That Keep the Gut’s Immune System in Check</i>	64
<i>How What You Eat Can Affect How Much You Eat</i>	50	The Inflammatory Bowel Disease Genetics Consortium	66
<i>Tracking Both the What and the When of the Human Diet</i>	51	Pancreatitis Research	67
Bariatric Surgery Research	52	<i>Chronic Pancreatitis: Cause, Not Gender, Determines the Disease</i>	67
<i>Weight Loss and Health Benefits from Bariatric Surgery in Teens with Severe Obesity</i>	52	<i>Identifying Risk Factors for Pancreatitis in Children</i>	67
<i>Weight-loss Surgery Contributes to Type 2 Diabetes Remission</i>	53	Irritable Bowel Syndrome Research	68
Obesity Research Workshops	54	<i>Pain Expectations: Altered Brain Responses in People with Irritable Bowel Syndrome</i>	68
DIGESTIVE DISEASES AND NUTRITION	57	<i>New Insights into Early-life Influences on Irritable Bowel Syndrome</i>	69
Gut Microbes in Health and Disease.....	59	<i>Adverse Childhood Events Associated with Irritable Bowel Syndrome</i>	69
<i>Nourishing Gut Bacteria To Help Young Children Avoid Undernutrition</i>	59	Insights into Acute Liver Failure Outcomes	70
<i>Early-life Exposures Affect Infant Health</i>	60	<i>Improved Outcomes and Survival Following Acute Liver Failure in Recent Years</i>	70
<i>Eating Fiber for the Health of Future Generations</i>	61		

Understanding and Treating Liver Disease	71	<i>Decline in Nephron Numbers in the Kidney Even in Healthy Aging</i>	94
<i>Toxin Provides Clues to Disease</i>		<i>Understanding the Role of an Important Family of Genes in Human Kidney Development.....</i>	95
<i>Processes Underlying Biliary Atresia</i>	71	<i>Technological Improvements Help Transplanted Kidney Tissue Connect with a Host’s Circulatory System</i>	95
<i>Controlling Levels of Bile Acids for Liver Health</i>	72	Kidney Stone Treatment.....	96
Insights into the Development of Liver Cancer.....	72	<i>Moving Stones with Sound—New Ultrasound Technology Repositions Kidney Stones in People</i>	96
<i>Key Biomarker Involved in Liver Cancer Development from Multiple Causes.....</i>	72	<i>New Approach May Reduce Risk of Kidney Stone Formation</i>	96
Workshops: Functional Bowel Disorders and Chronic Pancreatitis in the 21st Century	74	Kidney Transplantation	97
Story of Discovery: Illuminating the Inner World of the Gut Microbiome and Its Impacts on Human Health	75	<i>Promising Result Reported from Multi-center Kidney Transplantation Study.....</i>	97
Scientific Presentation: Dr. Lee Kaplan— Molecular Mechanisms Underlying the Beneficial Effects of Bariatric Surgery.....	81	Insights into Urological Pain Syndromes	98
Patient Profile: One Man’s Experience Surviving Acute Liver Failure	85	<i>Evaluating Nervous System Involvement in Women with Chronic Pelvic Pain</i>	98
KIDNEY, UROLOGIC, AND HEMATOLOGIC DISEASES	91	<i>Unique Microstructural Changes in Brains of People with Urologic Pain Syndromes Revealed.....</i>	98
Kidney Formation and Function in Health and Disease	93	Understanding Hematological Diseases	99
<i>Mining the Genome for Insights into Kidney Function and Development</i>	93	<i>Revving Up Human Red Blood Cell Production</i>	99
<i>A Cause of Hardening and Narrowing of Arteries in Kidney Disease Identified</i>	93	<i>The Double-edged Sword of a Pro-inflammatory Protein.....</i>	100

*Ramping Up Fetal Hemoglobin Production—
Implications for Red Blood Cell Diseases 100*

The Chronic Renal Insufficiency Cohort Study 102

Story of Discovery: Delineating the Anatomy
of the Developing Genitourinary Tract 104

Scientific Presentation: Dr. Joseph Bonventre—
Kidney Repair and Regeneration Shop 106

Patient Profile: Paying It Forward—
A Physician-scientist with Type 1 Diabetes
Participates in a Clinical Trial To Prevent
Kidney Disease 110

**EXTRAMURAL FUNDING TRENDS AND
SUPPORT OF CORE VALUES 115**

ACKNOWLEDGMENTS

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; 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/bladder pain syndrome and prostatitis; and hematologic diseases.

The 17th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- That controlling blood glucose early in the course of type 1 diabetes preserves heart health and reduces premature death
- A method for using cells from people with type 1 diabetes to make new β (beta) cells, for potential use in treating their disease
- That there are subtypes of β cells with differing properties, and the balance between them shifts as type 2 diabetes progresses
- Identification of a molecule produced during exercise that promotes physical endurance
- An insight into the pathogenesis of cystic fibrosis that may lead to improved approaches for preventing debilitating lung infections
- Multiple discoveries about ways the brain regulates appetite and digestion
- Explorations into potential new directions in obesity treatment
- How both food choices and timing may influence how much we eat
- Multiple revelations about how children's gut microbiomes are affected by factors such as nutrition, antibiotics, and Crohn's disease treatments
- Advancing our understanding of brain responses associated with irritable bowel syndrome and of the impact of stress early in life on this painful condition

- A better understanding of the etiology of pancreatitis in women and in children
- Understanding causes of liver disease and identifying pathways that could be therapeutically targeted to improve liver health
- Understanding artery hardening during chronic kidney disease and kidney deterioration during healthy aging
- Treating kidney stones with novel ultrasound technology, and potentially preventing them by a gene-silencing approach
- That kidney transplants from tissue-non-compatible live donors can improve survival compared to remaining on a donor waiting list or receiving a transplant from a tissue-compatible deceased donor

In addition to reporting on recent advances, this publication traces the multi-step path to research achievements through several “Stories of Discovery” and “Scientific Presentations.” These essays illustrate how major new discoveries that have greatly advanced biomedical science and are benefitting human health often emerge from many incremental insights gained from research investments spanning many years and even multiple research disciplines.

This report also includes personal stories of those who have given time and effort to participate in NIDDK-sponsored clinical research or whose lives have been transformed by biomedical research. Sisters with type 1 diabetes share their enthusiasm for an artificial pancreas, like the ones they helped test. A man tells his story of survival despite acute liver failure brought on by Reye syndrome. A physician-scientist describes his own participation in a trial to prevent diabetic kidney disease. A man traces how a lifetime in public health extended naturally into his own participation in a trial to determine how best to treat type 2 diabetes.

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

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

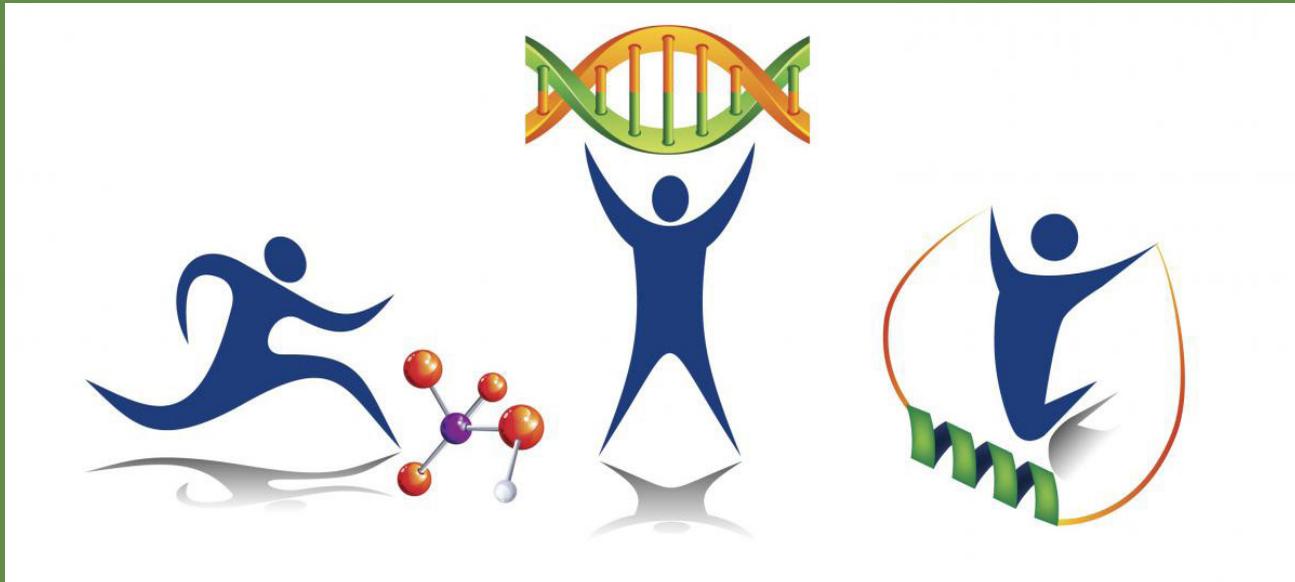
I invite you to visit us at www.niddk.nih.gov Health information, news, and scientific advances related to NIDDK research are also available on our Twitter feed: [@NIDDKgov](https://twitter.com/NIDDKgov)

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

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



Scan the QR code
with your mobile
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NIDDK website at
www.niddk.nih.gov



The health benefits of exercise are well documented, but it is not understood what molecular changes are induced by physical activity or how these changes improve the function of different tissues and organs in the body to promote health. As described in this chapter, the Molecular Transducers of Physical Activity Consortium is a trans-NIH effort to build an extensive catalogue of biological molecules affected by exercise in people, construct a comprehensive molecular map of these changes, and characterize the functions of these key molecules. By doing so, researchers hope to link the exercise-induced molecular changes to the advantageous effects of physical activity. By determining how physical activity improves health and prevents disease at a molecular level, scientists can discover new therapeutic targets and develop new approaches to personalized exercise medicine.

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.

This chapter provides a few examples of the Institute's commitment to basic and applied research relevant across a broad spectrum of scientific disciplines. For example, features in this chapter highlight the research of NIDDK-funded investigators who have won the distinguished PECASE early career award, an NIDDK workshop on gastrointestinal and urologic care simulation technology, and a trans-NIH program, co-led by the NIDDK, to determine what molecular factors mediate the benefits of exercise. Another feature describes the NIDDK's efforts to harness precision medicine to advance human health and develop more personalized therapies for diseases within the NIDDK's mission.

UNDERSTANDING MITOCHONDRIA'S RESPONSE TO STRESS

Stressed to the Breaking Point—How a Cell's Mitochondria Respond to Stress To Maintain Health: Scientists identified a key process for how mitochondria, the cell's energy generators, or powerhouses, handle metabolic stress. Mitochondria are referred to as the "powerhouses" of the cell because they take energy that is ingested in the form of sugars or fats and convert it to fuel for the cell.

Unhealthy mitochondria have been implicated in many disorders and diseases, including diabetes.

Cells typically contain many mitochondria, in some cell types up to several thousand. Mitochondria are dynamic, which allows them to maintain appropriate shape, size, and number. They are continually joining with one another (fusion) or splitting into smaller units (fragmentation or fission) in response to changing conditions. Metabolic stress, for example, induces mitochondria to undergo fragmentation; however the links between stress and the fission machinery have been poorly understood.

In a new study, scientists investigated whether a central metabolic sensor—adenosine monophosphate activated protein kinase (AMPK)—plays a role in triggering mitochondrial fragmentation due to metabolic stress. They found that treating a human cell line originally from bone tissue with chemicals that stress the mitochondria resulted in extensive fragmentation and activated AMPK. Genetically disrupting AMPK prevented fragmentation. Thus, the chemicals cannot trigger mitochondrial fission in cells that lack AMPK. To demonstrate further that AMPK provides a key signal for fragmentation, the researchers directly activated AMPK and found that this was sufficient to induce

mitochondrial fragmentation, even in the absence of the chemicals. They also activated AMPK in mouse cells with similar results, indicating that this function of AMPK is conserved.

Through additional experiments, the scientists found that AMPK triggers fission by activating a protein called MFF, which then recruits another protein (DRP1) to the membrane of mitochondria to induce fragmentation. They speculate that this process may be an important way for cells to rid themselves of damaged mitochondria. Additional research into the role that AMPK plays in regulating mitochondria may provide new insights into how mitochondrial stress affects metabolic diseases and disorders.

Toyama EQ, Herzig S, Courchet J,...Shaw RJ. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. Science 351: 275-281, 2016.

INSIGHTS INTO WOUND REPAIR

The Ties That Bind in Tissue Scaffold Assembly:

Scientists have defined an important role for chloride ions in the formation of the molecular scaffolding that provides structure and biological function in tissues throughout the body. Cells in many tissues are surrounded by a thin layer called a basement membrane (BM). These molecular scaffolds consist of proteins and chemicals that provide structural integrity and play a variety of functional roles in normal cellular biology and disease. For example, disruption of the collagen IV protein network in kidney BM can lead to a condition known as Alport's syndrome, in which patients suffer from reduced kidney function and eventual kidney failure.

The assembly of collagen IV networks initially involves a two-step process. First, within cells, three individual, long, thin collagen protein chains wind around each other to form a triple-helical structure called a protomer, in which the tips on one end of each chain interact through a segment called an

NC1 domain. Second, two separate protomers that are released from the cell bind together through these domains, forming an NC1 hexamer. Additional molecular steps then lead to the establishment of the collagen IV network in the BM.

To better understand how these complex collagen IV networks assemble, a team of scientists isolated the individual collagen protein chains and established conditions under which they spontaneously form protomers in the laboratory. The researchers tested different ions normally present in the BM and found that chloride ions were required for NC1 hexamer assembly; none of the other ions tested could promote these interactions at physiologically relevant concentrations. Using known information about the physical structure of NC1 domains, the scientists performed computer simulations to determine the effects of chloride ion interaction with specific amino acids (individual building blocks that link to form proteins) in the NC1 domains. The simulations predicted that binding of a chloride ion triggers changes in the shape of NC1 domains, thereby encouraging assembly. This prediction was confirmed when the researchers mutated these critical amino acids, which are largely conserved in collagen IV proteins across the animal kingdom, to prevent chloride binding, and the NC1 domains could no longer interact. They also examined the role of chloride in the BM of cells in culture, finding that chloride was required for collagen IV assembly under these more physiological conditions, not just in a test tube.

Together, these data uncover a role for chloride ions as signals that promote the assembly of collagen IV networks by inducing critical structural changes in NC1 domains that allow protomers to interact. This study could help provide a foundation of knowledge to develop strategies to prevent or treat diseases caused by dysfunctional BMs.

Cummings CF, Pedchenko V, Brown KL,...Hudson BG. Extracellular chloride signals collagen IV network assembly during basement membrane formation. J Cell Biol 213: 479-494, 2016.

Dr. Gary Felsenfeld Receives Horwitz Prize: NIDDK Intramural Research Program Scientist Pioneered the Field of Epigenetics



Dr. Gary Felsenfeld

Dr. Gary Felsenfeld, a senior investigator in NIDDK's Intramural Research Program and an NIH Distinguished Investigator, is a 2016 recipient of the Louisa Gross Horwitz Prize. Columbia University gives the award to

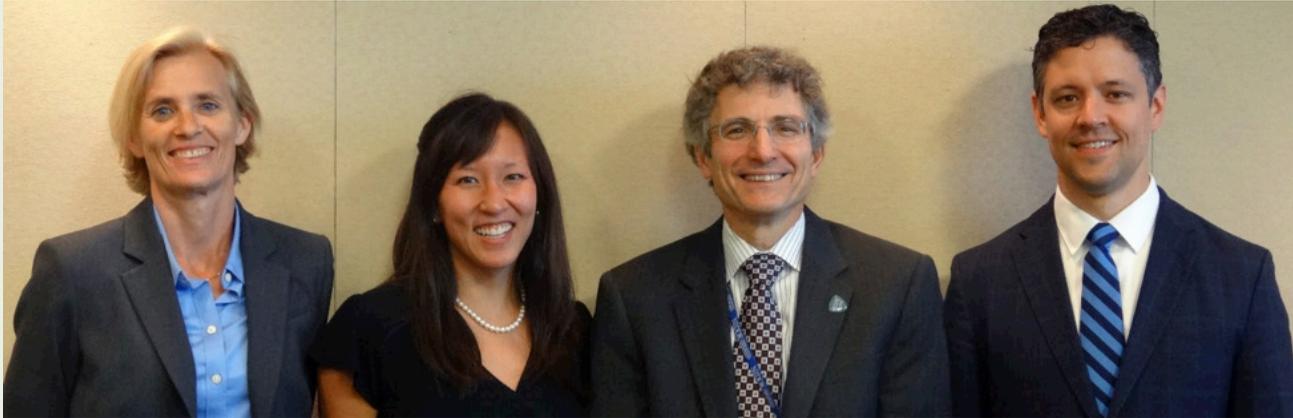
recognize outstanding basic research in biology or biochemistry. Since the prize was first awarded in 1967, 43 awardees have gone on to win Nobel Prizes.

Dr. Felsenfeld's research has focused on how proteins that bind DNA in the cell's nucleus alter the structure and chemical nature of DNA. These interactions affect whether and when genes are "turned on or off," leading to the exquisite regulation of the cell's activities. Understanding the changes in these protein-DNA interactions that are associated with both normal and abnormal growth and development is essential to advancing progress in diseases such as diabetes and cancer.

Dr. Felsenfeld's pioneering work helped lead to the formation of this field of research, called "epigenetics." He has been a member of the NIDDK Intramural Research Program's Laboratory of Molecular Biology since 1961. In addition to his extraordinary research, Dr. Felsenfeld is an accomplished mentor, having trained numerous scientists who have gone on to distinguished research careers.

(Information adapted from original article by Krysten Carrera, published on September 23, 2016 in the NIH Record.)

NIDDK-supported Scientists Receive Presidential Award



Left to right: Dr. Forsberg, Dr. Tye, NIDDK Deputy Director Dr. Germino, and Dr. Pagliarini.

Three scientists supported by the NIDDK have received the Presidential Early Career Award for Scientists and Engineers (PECASE) in 2016. The PECASE is awarded annually to scientists and engineers who, while early in their research careers, have pursued innovative research and shown outstanding scientific leadership. Among the recipients were three NIDDK extramural grantees—Camilla Forsberg, Ph.D., David J. Pagliarini, Ph.D., and Kay Maxine Tye, Ph.D.

Dr. Forsberg, a Professor of Biomedical Engineering at the University of California, Santa Cruz, received a PECASE award in recognition of her work to understand the mechanisms that regulate stem cell fate decisions. Her studies on hematopoietic stem cells (which give rise to all other blood cells) concentrated on finding new insights into how the blood and immune system is established and may guide strategies for combatting blood disorders.

Dr. Pagliarini, an Associate Professor of Biochemistry at the University of Wisconsin-Madison and Director

of the Metabolism Research Group at the Morgridge Institute for Research, received a PECASE award for his investigations into mitochondrial proteins and the development of disease. His research focuses on the types of mitochondrial dysfunction that can contribute to conditions such as type 2 diabetes and obesity.

Dr. Tye, an Assistant Professor at the Picower Institute for Learning and Memory in the Department of Brain and Cognitive Sciences at the Massachusetts Institute of Technology, received a PECASE award in honor of her studies to develop and apply new technologies to address compulsive sugar intake. Her research explores the brain activity involved in unhealthy eating choices and habits that can lead to obesity.

In addition to the NIDDK-supported recipients, 17 other scientists supported by the NIH received the PECASE for their scientific achievements. The NIH has funded 253 PECASE recipients since the award's inception in 1996.

The PECASE is the most prestigious award given in the United States to scientists at the outset of their independent research careers. These awards support the continued professional development of awardees, promote careers, 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: <http://grants.nih.gov/grants/policy/pecase.htm>

Precision Medicine—Moving Away from “One Size Fits All” Medical Treatments



Contrary to its name, a “one size fits all” clothing garment will not actually fit everyone. It may be a perfect fit for some, but it will be too big or too small on others. It is not made with each individual in mind.

The same could be said for most of today’s medical treatments. Most treatments are also “one size fits all,” which may work well for some people but not for others. Just as clothing fits better when a tailor alters it to fit the individual, medical treatments could also work better if they were tailored to the person. That is the goal of precision medicine—to move away from a “one size fits all” approach so that health care decisions are personalized and based on individual variability in genes, environment, and lifestyle. Precision medicine would take into account these differences between individuals and enable health care that predicts more accurately which treatment and prevention strategies will work in specific people.

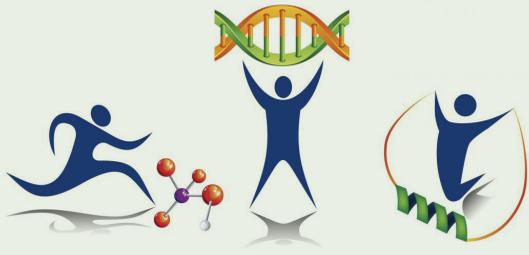
Already, precision medicine has some applications across a number of diseases. To extend the benefits of precision medicine to more diseases, the NIH launched the *All of Us* Research ProgramSM as a part of the Precision Medicine Initiative[®]. *All of Us* involves building a national research cohort of 1 million or more U.S. volunteers who broadly reflect the diversity of the country’s population; cohort recruitment started in 2016. Using information and biological samples provided by the cohort, researchers will

begin teasing out how genetics, environment, lifestyle, and other factors contribute to an individual’s health and disease, including diseases and disorders within the NIDDK mission. More information about the *All of Us* Research Program is available at: www.nih.gov/research-training/allofus-research-program

Complementing the *All of Us* Research Program, the NIDDK also supports research toward precision medicine. For example, an ongoing clinical trial, called GRADE, is comparing the long-term benefits and risks of four widely used diabetes drugs in combination with metformin for treating people with type 2 diabetes. Results are expected to shed light on factors that are associated with response to and failure of the different treatments, which could promote personalized therapy. Additionally, analysis of extensive genetics data from the Inflammatory Bowel Disease (IBD) Genetics Consortium contributed to a more precise classification of the different types of IBD. Knowing what type of IBD a person has could eventually help health care providers offer personalized treatments. A new Kidney Precision Medicine Project aims to build on recent observations that chronic kidney disease (CKD) and acute kidney injury (AKI) are not single, uniform diseases. Defining subgroups of CKD and AKI—and understanding underlying molecular pathways—can facilitate identification of specific drug targets and enable individualized care.

Knowledge stemming from these and other NIDDK- and NIH-supported research efforts can move health care away from a current “one size fits all” approach for most medical treatments and enable individualized prevention and treatment strategies for more people.

Molecular Transducers of Physical Activity



The health benefits of exercise are well documented; scientists have shown that regular physical activity contributes to improved cardiovascular and respiratory health, insulin sensitivity, muscle strength, and mood. However, the precise molecular changes that occur in response to physical activity and the effects these changes have on different tissues and organ systems are poorly understood.

The NIH convened a meeting in October 2014 to identify knowledge gaps and research opportunities that address these questions, and to deliberate ways in which to catalyze coordinated strategies for progress. This meeting and subsequent discussions with the research community helped guide the creation of the Molecular Transducers of Physical Activity Consortium. The consortium is managed by several institutes at the NIH, including the NIDDK, and sponsored by the NIH Common Fund. The consortium's investigators aim to build an extensive catalogue of biological molecules affected by exercise in people, construct a comprehensive molecular map of these changes, and characterize the functions of these key molecules. The program also aims to create a user-friendly database that researchers can access to aid studies into how exercise promotes better health and prevents disease.

To ensure a broad scope of study, participants will be equally distributed between males and females and will include a variety of ages, racial and ethnic groups, and fitness levels. Throughout the course of the study, both active and sedentary participants will perform different types of exercise, and blood and tissue samples will be collected and analyzed extensively to identify a variety of biological molecules. These analyses will allow for characterization of molecules that are altered at different times during and after physical activity and that could mediate effects on overall health.

In addition to human studies, corresponding studies will be conducted in animal models in order to gather data from cells and tissues affected by physical activity that are not easily studied in humans, such as lung, heart, and brain. The combination of human and animal data will allow researchers to determine how physical activity affects specific molecules in specific tissues at certain times throughout an exercise regimen.

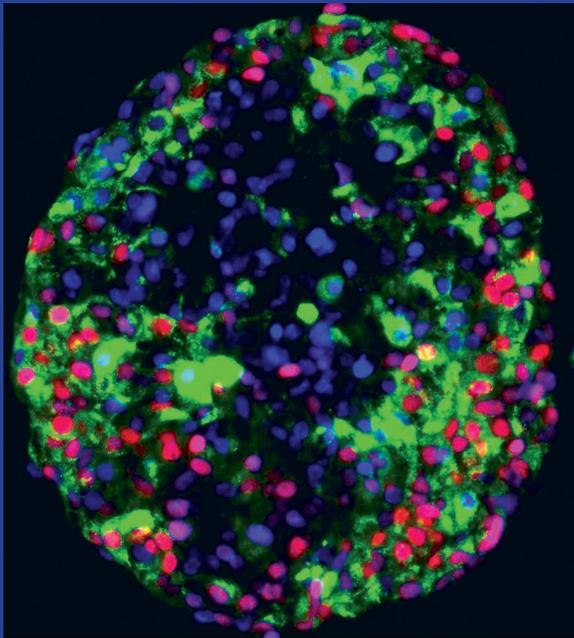
The goal of this program—to determine how physical activity improves health and prevents disease at a molecular level—may contribute to the discovery of new therapeutic targets and could help to provide new approaches to personalized exercise medicine.

For more information on the consortium, please see:
[https://commonfund.nih.gov/
MolecularTransducers/](https://commonfund.nih.gov/MolecularTransducers/)

Workshop on Opportunities for Simulation Research in Gastrointestinal and Urologic Care

On June 10, 2016, the NIDDK and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) co-sponsored a workshop on "Simulation Research in Gastrointestinal and Urologic Care: Challenges and Opportunities," held on the NIH campus in Bethesda, Maryland. The quality of clinical care available to diagnose and treat gastrointestinal (GI) and urologic diseases depends largely on the expertise of the clinician performing a given procedure, such as an endoscopy or surgery. Like the use of a flight simulator to train airline pilots, training to develop expertise in performing a medical procedure with a successful outcome can be greatly enhanced through use of a simulated experience, which can consist of a computer application, a mannequin or other model, or even a completely simulated surgical environment. Simulation can also help to reduce the number of medical errors made while clinicians are developing a particular procedural skill by lessening their need to "practice" on real patients. Additionally, the use of simulation has the potential to develop the ability to make care more personalized, by allowing a training model to incorporate elements of a person's unique disease and surgical needs. However, adoption of simulations by training programs for medical personnel has been limited.

The purpose of the workshop was to explore research opportunities for enhancing the adoption of simulation applications by clinicians caring for people with GI and urologic conditions. Presentations by clinicians and scientists in academia and the federal government, as well as others with experience in non-medical fields such as music and sports, highlighted the use of simulation for optimizing training and learning new skills. The speakers also identified recent advances in the field, such as applications for military medicine and the production of more realistic tissue and mannequins, using technologies such as three-dimensional printing. Following the presentations, participants discussed research needs during breakout groups and reported on the groups' recommendations for addressing gaps and opportunities. A summary of the workshop and its research recommendations has been disseminated broadly throughout the scientific community via publications in two academic journals in the medical literature. The workshop's recommendations are expected to inform future collaborations by the NIDDK and the NIBIB to address these priorities for enhancing use of simulation in GI and urologic care.



In people with type 1 diabetes, insulin-producing β (beta) cells in the pancreas are destroyed. Without insulin, the tissues of the body cannot absorb or use glucose (sugar), the major cellular fuel. Scientists are therefore pursuing strategies to replace the destroyed β cells and restore insulin production, including by transplantation of β cells produced in the laboratory. In research described in this chapter, scientists generated functional β cells in the laboratory from the skin cells of people with type 1 diabetes by first reprogramming the skin cells to become stem cells, and then coaxing the stem cells to become β cells. This image depicts a mixed cell population composed of mature stem cell-derived, insulin-producing β cells (stained

green or both green and red), cells that may be on their way to become insulin-producing β cells (stained red only), and cells that do not belong to the β cell lineage (stained blue). While many research questions remain before a stem cell-derived β cell transplant procedure will be ready for testing in humans, this advance is a significant step forward toward developing cell replacement therapy for type 1 diabetes.

*Image courtesy of Dr. Douglas Melton, Harvard University, and Dr. Jeffrey Millman, Washington University. Originally published in Millman JR, Xie C, Van Dervort A, Gürtler M, Pagliuca FW, Melton DA, *Nat Commun* 7: 11463, 2016, available at: www.nature.com/articles/ncomms11463 Image copyright 2016 from Millman, et al., and reprinted under a Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>).*

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 two decades, and the rate of new cases of diagnosed diabetes has begun to fall, disease burden remains significant as the number of people with diabetes is still very high.^{2,3} Diabetes can affect many parts of the body and is associated with serious complications, such as heart disease and stroke, blindness, kidney failure, and lower-limb amputation. In addition to these human costs, the estimated total financial cost for diabetes in the United States in 2012—including costs of medical care, disability, and premature death—was \$245 billion.⁴ Effective therapy can prevent or delay diabetic complications, but up to one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.³

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 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

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 Centers for Disease Control and Prevention. *Diabetes—working to reverse the U.S. epidemic, at a glance 2016*. Atlanta, GA: U.S. Department of Health and Human Services, 2016.

⁴ American Diabetes Association. *Diabetes Care* 36: 1033-1046, 2013.

eye, kidney, nerve, and heart complications associated with type 1 diabetes. However, despite vigilance in disease management, with current technologies to test blood glucose levels and administer insulin, it is still not possible for people with type 1 diabetes to control blood glucose levels as well as functional pancreatic β cells do. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery. In this regard, a milestone was achieved this past year when the U.S. Food and Drug Administration approved the first commercial “hybrid artificial pancreas” device that automatically links glucose monitoring and insulin delivery. The NIDDK supported early research that contributed to the development of the approved device and continues to support research to test and improve artificial pancreas technologies. Researchers are also working to develop β cell replacement therapies, such as islet transplantation, to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.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.¹ Type 2 diabetes occurs at higher rates among racial and ethnic minority populations in the United States, including African Americans, Hispanic and Latino Americans, American Indians, some Asian Americans, and Native Hawaiians and Pacific Islanders.¹ Gestational diabetes is also a risk factor: about half of women with gestational diabetes will develop type 2 diabetes within 5 to 10 years after giving birth.⁵

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not respond to insulin properly. 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 elevated risk of developing type 2 diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with prediabetes can dramatically reduce their risk of developing type 2 diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight. To a more limited degree, the safe and well-tolerated drug metformin can also help prevent or delay type 2 diabetes. Moreover, follow-up research has shown that the benefits of reduced diabetes risk from weight loss or metformin can persist for at least 15 years.

Type 2 diabetes was previously called “adult-onset” diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects youth from racial and ethnic minority populations in the United States. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. For example, 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

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

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.

TYPE 1 DIABETES—HEALTH BENEFITS OF GOOD GLUCOSE MANAGEMENT

Intensive Blood Glucose Management for Those with Type 1 Diabetes Preserves Heart Health and Reduces Risk of Early Mortality:

A long-term NIDDK study reports that keeping blood glucose (sugar) as close to normal as possible for an average of 6.5 years early in the course of type 1 diabetes reduces cardiovascular (heart and blood vessel) disease and can reduce mortality to rates close to those seen in people of similar age in the general population. The landmark Diabetes Control and Complications Trial (DCCT) began in 1983. The DCCT randomly assigned half its participants to an intensive blood glucose management regimen designed to keep blood glucose levels as close to normal as safely possible, and half to the less intensive conventional treatment at the time. When DCCT ended in 1993, it was clear that intensive management had significantly reduced eye, nerve, and kidney complications, but at that time the participants were too young to determine their rates of cardiovascular disease. All DCCT participants were taught the intensive management regimen and invited to join the Epidemiology of Diabetes Interventions and Complications (EDIC) study. EDIC continued to monitor participants' health, and overall blood glucose management has since been similar in both DCCT treatment groups.

To study the long-term effects of the different treatments tested in the DCCT, researchers examined differences in cardiovascular problems, which can take many years to develop, between the former intensive and conventional treatment groups. After an impressive average 30-year follow-up, DCCT/EDIC researchers found that those who practiced intensive blood glucose management during the DCCT still had significantly reduced cardiovascular disease compared to those who did not, despite having similar

blood glucose management for 20 years after the DCCT ended. Compared to the former conventional treatment group, the former intensive management group had a 30-percent reduced incidence of cardiovascular disease and 32 percent fewer major cardiovascular events (such as non-fatal heart attack, stroke, or death from cardiovascular disease) after 30 years of follow-up. These results were similar for both men and women who participated in the studies. However, the beneficial effects of intensively managing blood glucose during the DCCT appeared to be wearing off over time. For example, after 20 years of follow-up, DCCT/EDIC researchers reported that the former intensive treatment group had a 42-percent reduced risk of cardiovascular disease compared to the former conventional treatment group. After 30 years of follow-up, that number had fallen to 30 percent. Even with this reduction in protection, these new data show that a finite period of near-normal blood glucose management early in the course of type 1 diabetes can have beneficial effects on cardiovascular health for up to 30 years.

Historically, those with type 1 diabetes have had a higher mortality rate than the general population. Previous DCCT/EDIC analyses compared intensive versus conventional blood glucose management and showed that those in the former intensive treatment group had reduced mortality compared with that of the former conventional treatment group. Now, mortality in the DCCT/EDIC study from its inception through 2014 was compared to 2013 national mortality data. Researchers found that overall mortality when both DCCT/EDIC treatment groups were combined was no greater than what would be expected in the general U.S. population. However, they found that the mortality rate in the former conventional treatment group was 31 percent higher than that seen in the general population. While the former intensive treatment group's mortality rate was below that in the general population, the difference was not statistically significant. Researchers also found participants' long-term blood glucose control affected mortality rates, and those who had worse control had correspondingly worse mortality rates. This effect of blood glucose control on lifespan was more pronounced among women than among men. In general, these results suggest that the increased mortality historically seen in those with type 1 diabetes can be reduced or eliminated through careful management of blood glucose.

Overall, these findings add to DCCT/EDIC's decades of evidence demonstrating how people with type 1 diabetes can dramatically increase their chances of living long, healthy lives by practicing early, intensive blood glucose management.

Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes: the DCCT/EDIC study 30-year follow-up. [Diabetes Care](#) 39: 686-693, 2016.

Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group. Mortality in type 1 diabetes in the DCCT/EDIC versus the general population. [Diabetes Care](#) 39: 1378-1383, 2016.

BETA CELLS AND DIABETES

Making Beta Cells from People with Type 1 Diabetes:

Scientists generated functional β (beta) cells from skin cells of people with type 1 diabetes. In type 1 diabetes, a misguided attack by the immune system leads to destruction of insulin-producing β cells found in clusters called islets in the pancreas. Although administration of insulin *via* injections or a pump is life-saving, it does not mimic the exquisite blood glucose (sugar) control of the pancreas. Therefore, scientists are pursuing strategies to replace the destroyed β cells. One way to do that is through islet transplantation—an experimental procedure using islets from a cadaveric donor. The procedure has shown promise for people with difficult-to-control diabetes, but has significant challenges: donor islet tissue is limited, and immunosuppressive medications, which have toxic side effects, are required to prevent rejection of tissue transplanted from another individual. Toward overcoming the first barrier, scientists recently developed a new laboratory production method to make large quantities of β cells—called stem cell-derived β (SC- β) cells—from human stem cells. This method could, with further development, be used to make β cells from a sample of cells from a person with type 1 diabetes in the quantities needed for transplantation back into that same person. These cells would likely require protection from the autoimmune attack, but might not require toxic immunosuppressive medications to prevent rejection of the tissue.

To investigate this possibility, in new research, scientists used skin cells from three people with type 1 diabetes (T1D cells) and three people without diabetes (ND cells). By introducing specific factors into these cells and using the new large-scale production method they developed, they made the skin cells become stem cells—cells that could subsequently become any cell type. They then, by introducing other factors, coaxed these stem cells to become SC- β cells (T1D SC- β cells and ND SC- β cells). Cells from the two different origins showed no differences in the ability to become SC- β cells, indicating for the first time that cells from a person with type 1 diabetes could be used to make SC- β cells.

Next, the scientists demonstrated that the T1D SC- β cells functioned like healthy β cells. For example, in laboratory culture, T1D SC- β cells secreted insulin in response to glucose; they also released insulin in response to diabetes drugs that are known to stimulate insulin secretion, demonstrating their potential for use in screening for new diabetes drugs. The T1D SC- β cells also functioned in live animals: when T1D SC- β cells were transplanted into male mice, they produced insulin in response to glucose and controlled the animals' blood glucose levels.

Many research questions remain before an SC- β cell transplant procedure will be ready for testing in humans. First, it remains possible that differences between T1D SC- β and ND SC- β cells could appear over a longer time period than in the study. Second, it is not known how the T1D SC- β cells will interact with the recipient's immune system; for example, it is not yet clear whether these cells could still be rejected, even though they were derived from the recipient's own cells; and it is likely that these cells would be subject to the same autoimmune attack that destroyed the person's original β cells. Third, individual differences in type 1 diabetes may affect the production, function, or transplant success of T1D SC- β cells. Thus, further research will illuminate the potential of T1D SC- β cells as a therapy for type 1 diabetes. Nonetheless, these results mark another significant step forward toward a cell therapy for type 1 diabetes, and also provide a valuable resource for drug screening and studying the development of the disease.

Millman JR, Xie C, Van Dervort A, Gürtler M, Pagliuca FW, and Melton DA. Generation of stem cell-derived β -cells from patients with type 1 diabetes. [Nat Commun](#) 7: 11463, 2016.

New Biomaterial Protects Transplanted Insulin-producing Beta Cells from the Immune System:

Scientists have developed a new biomaterial that can protect transplanted β (beta) cells and allow them to function for months in a mouse model of type 1 diabetes without the need for immunosuppression. Transplantation of organs or cells from one person (or animal) to another usually requires the recipients to take immunosuppressive drugs to prevent their immune systems from attacking and rejecting the transplant. Such an immune attack can lead to scar tissue formation (called fibrosis) around the transplant, and eventual death of the transplanted material. Immunosuppressive drugs, however, carry their own serious risks and side effects, and a method to protect transplanted tissues without immunosuppression would greatly benefit people with many diseases. One of these diseases is type 1 diabetes, in which the insulin-producing β cells are destroyed by a misguided immune attack. Transplanting lab-grown β cells into people whose own β cells are not functioning properly is a promising experimental treatment for this disease. Researchers are developing methods for large-scale, laboratory production of β cells that release insulin in response to elevated glucose (sugar) levels. However, the misguided immune response that destroyed the β cells of a person with type 1 diabetes may also attack transplanted β cells. Thus, to realize β cell transplantation's potential fully, it is important to identify ways to protect the transplanted cells from the host's immune system without immunosuppression.

One candidate biomaterial being tested for its transplant-protecting properties is a low-toxicity, inexpensive material called alginate. Previous alginate compounds were only able to protect transplanted β cells and enable them to regulate hosts' blood glucose levels for short periods, as the alginate elicited significant immune responses, fibrosis around the transplants, and eventual death of the transplanted cells. Scientists hypothesized that varying the chemistry of alginate might create an alginate variant that could protect transplanted tissues without provoking a strong immune response. To test this idea, the researchers generated a library of chemically altered alginate variants, and evaluation of the library revealed variants that produced substantially reduced immune reactions and fibrosis when tested in rodents and non-human primates. However, could the new biomaterials protect living, transplanted tissue?

To test this, researchers asked whether or not the most promising new alginate variant (called TMTD alginate) could protect lab-grown β cells against immune attack in a male mouse model of type 1 diabetes in which β cells had been destroyed with a chemical. They found that transplanted β cells encapsulated in TMTD alginate caused a weaker immune response and significantly less fibrosis than cells encapsulated in other alginate compounds. The encapsulated β cells were able to respond to and regulate the mice's blood glucose levels in a normal range. This "cure" of the mice's diabetes lasted until researchers removed the transplanted cells after 174 days, during which the alginate protected the human β cells from immune system attack without the need for immunosuppression. After 174 days, the TMTD alginate-coated transplant capsules still contained living β cells, still produced insulin, and had caused only minimal fibrosis at the implantation site.

Further research is needed to determine how well TMTD alginate can protect various types of transplanted materials in people. Additionally, more research is needed to determine how well TMTD alginate can protect β cells transplanted into mice or people with type 1 diabetes, where there is an ongoing misguided immune attack against the β cells. Given these hurdles, this new biomaterial's ability to protect transplanted β cells in mice with chemically induced type 1 diabetes is a significant step forward in developing a long-term cellular therapy for this disease.

Vegas AJ, Veisoh O, Doloff JC,...Anderson DG. Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates. *Nat Biotechnol* 34: 345-352, 2016.

Vegas AJ, Veisoh O, Gürtler M,...Anderson DG. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat Med* 22: 306-311, 2016.

Newly Discovered Proteins in Beta Cells Are Targets for Autoimmune Attacks Implicated in Type 1 Diabetes:

Researchers have found that immune cells can target naturally occurring fused protein fragments found in β (beta) cells, a discovery that may explain how the type 1 diabetes autoimmune attack is initiated and open up new disease treatment and prevention opportunities. Type 1 diabetes is caused by the immune system launching a misguided attack that destroys the insulin-producing β cells in

the pancreas. Immune cells called T cells are thought to participate in this attack, and determining what proteins they target—and how the attack might be prevented—is a subject of keen interest. Studies of T cell lines known to attack β cells in mice have identified several relevant T cell targets, including fragments of β cell proteins. However, some of the identified protein fragments are small and only weakly stimulate T cells, suggesting that they are only a part of the T cell's target.

Because fragments of the insulin protein also can trigger an immune response in type 1 diabetes, researchers hypothesized that “hybrid insulin peptides” (HIPs) made of insulin's C-peptide fragment fused to another immune-triggering protein fragment might be the T cells' true β cell target and elicit a stronger—and thus more damaging in people—response. To test this idea, researchers created a library of HIPs and tested their ability to stimulate an immune response in diabetes-causing mouse T cell lines. The researchers found that HIPs made of the C-peptide fragment and either of two naturally occurring fragments of other β cell proteins activated the diabetes-causing T cells over 10,000 times more strongly than the protein fragments alone. This observation supported the idea that T cells target the fusion proteins more strongly than the individual protein fragments. Further experiments determined that one of these HIPs was present in mouse β cell extracts and that HIP-reactive T cells were present in the pancreas and spleen in a female mouse model of type 1 diabetes. Thus, these HIPs are produced and recognized by T cells naturally in mice. The researchers also found T cells that react to similar HIPs (C-peptide fused to two different human β cell protein fragments) in the pancreata of two human males with type 1 diabetes. Whether these HIP-reactive T cells have a role in causing type 1 diabetes still needs to be determined.

Overall, this study has identified a novel class of insulin-fused targets for T cell attacks, which may be critical initiators of β cell destruction. Further research could determine whether these and other fusion proteins mediate the autoimmune attacks that cause type 1 diabetes and other autoimmune diseases, and whether this process can be slowed or halted to prevent disease.

Delong T, Wiles TA, Baker RL, ...Haskins K. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. Science 351: 711-714, 2016.

Not All Beta Cells Are Alike—Discovery Helps Explain Altered Insulin Secretion in Type 2 Diabetes:

Researchers have discovered that human pancreatic islets have four separate subtypes of β (beta) cells, and that islets from people with type 2 diabetes have abnormal percentages of the different subtypes. Human islets have long been known to have several distinct cell types, including β cells that release insulin in response to glucose. Until now, all β cells were thought to be alike. However, because previous research hinted at the possibility of functional differences among β cells, scientists sought to determine whether there are distinct types of β cells. To examine this question, the scientists developed novel antibodies—immune proteins that each bind and recognize only very specific structures—that can distinguish between different proteins present on the surface of β cells. This allowed them to sort β cells from islets from male and female donors. In this way, they were able to distinguish four separate β cell subtypes, which they designated β 1 through β 4, all of which proved able to produce insulin. The percentages of each subtype were similar in islets from 17 donors without diabetes; β 1 was generally the most abundant and β 4 was typically rarest. It was a much different picture, however, in islets from men and women with type 2 diabetes. For example, in islets from most people with type 2 diabetes, β 3 and β 4 cells were more abundant, and β 1 less abundant, than in people without the disease.

These observations led the researchers to wonder whether an altered distribution of β cell subtypes may contribute to the glucose control problems associated with type 2 diabetes. To address this, they examined whether the newly discovered subtypes functioned differently. Although they found that overall gene expression (how genes are “turned on” or “turned off”) was similar, some genes were indeed expressed at different levels in the subtypes, including genes known to play a role in type 2 diabetes and insulin secretion, suggesting functional differences among the subtypes. Importantly, the scientists also found that insulin secretion in response to glucose differed among the subtypes, with β 1 cells being the most glucose responsive. In contrast, β 4 cells had the

highest basal insulin secretion rate: that is, they secrete more insulin (although still not a lot) when glucose levels are low and insulin is not needed. These observations suggest that differences in the percentages of β cell subtypes might contribute to the altered timing of insulin secretion and poor glucose control seen in people with type 2 diabetes.

This research has shed important new light on β cell biology, showing that not all β cells are alike and that

the distribution of the four newly discovered β cell subtypes is altered in people with type 2 diabetes. Further research is needed to understand the origin of the subtype differences, as well as to determine whether these differences could be capitalized upon for type 2 diabetes treatment.

Dorrell C, Schug J, Canaday PS,...Grompe M. Human islets contain four distinct subtypes of β cells. [Nat Commun](#) 7: 11756, 2016.

The Human Islet Research Network: Toward Innovative Strategies for the Treatment and Prevention of Type 1 Diabetes



Type 1 diabetes is characterized by the loss of insulin-producing β (beta) cells in the pancreas. Replacing those lost β cells, which are part of clusters of cells called islets, could improve the health of people with the disease and reduce the significant burden associated with managing the disease. This, therefore, is an important goal of type 1 diabetes research. Decades of research, including by the now-concluded NIDDK-supported Beta Cell Biology Consortium, revealed key insights into how β cells develop and function, laying the path for cell-based therapies. To build on this success, in 2014 the NIDDK launched the Human Islet Research Network (HIRN), a new team-science program to pursue innovative strategies to protect and replace β cells in people with diabetes.

More than 80 scientists with diverse expertise belong to HIRN's four independent consortia working on different, but complementary, research goals using human cells and tissues. One consortium is focused on discovery of biomarkers of β cell injury that will be important for testing strategies to stop β cell destruction early in the disease process. Another is combining advances in generation of functional human pancreatic β cells with tissue

engineering technologies to develop micro-devices that will support functional human islet growth for transplantation. A third is developing approaches to model the interaction of the immune system and β cells in type 1 diabetes, and a fourth is investigating methods to increase or maintain functional β cell mass. A Coordinating Center and Bioinformatics Center aid HIRN investigators in sharing data and resources within the Network as well as with the broad scientific community to facilitate scientific interactions and accelerate research.

HIRN investigators have gotten off to a quick start, and several exciting research advances are described in this publication, including the discovery of four distinct subtypes of β cells and the generation of β cells using stem cells from people with type 1 diabetes. Additionally, HIRN has developed a website (www.hirnetwork.org) to provide detailed information about the Network, its investigators, projects, and progress.

More information about HIRN and other programs supported by the *Special Statutory Funding Program for Type 1 Diabetes Research* is described in a June 2016 report: <http://bit.ly/t1dreport>

COMBATING TYPE 2 DIABETES IN YOUTH

TODAY Study Helps Predict Whose Blood Glucose

Will Rise Tomorrow: A new study suggests an approach to help clinicians with adolescent patients who have type 2 diabetes distinguish whose diabetes is likely to remain adequately controlled with standard metformin therapy, and whose will need more careful monitoring and potentially more aggressively stepped-up treatment to stave off rapid disease progression. The Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial was the first major trial to test approaches to managing type 2 diabetes in the small but rapidly growing number of adolescents and young adults with the disease. The study showed that type 2 diabetes often progresses more rapidly in young people than in middle-aged and older people with the disease. By the end of the 4-year trial, the standard first-line drug metformin was insufficient to control blood glucose (sugar) adequately in about half of the participants. (Adequate blood glucose control was defined by the study as keeping participants' HbA1c—a measure of long-term blood glucose control—below 8 percent.) Treatment with both metformin and another medication, rosiglitazone, worked somewhat better than metformin alone. However, even this combination of medications failed to maintain adequate glucose control in a high proportion of the adolescents in the study. But interestingly, most of those whose diabetes was not well controlled by metformin (or metformin with rosiglitazone) saw their blood glucose rise outside the prescribed range very early. In fact, metformin had failed to maintain good blood glucose control in about one-fourth of the participants within less than 1 year. After that, fewer and fewer additional participants saw their blood glucose become too high. This suggests that while it is critical to find better means of controlling blood glucose in young people with type 2 diabetes, in many cases (like those whose blood glucose stayed in control for the entire study) the safe, inexpensive drug metformin will actually be sufficient. An important question then, is how can we tell which young people with diabetes are likely to need more aggressive treatment to control their blood glucose?

To address this, the authors of the current study compared characteristics of the group whose blood glucose remained in control during the TODAY trial with characteristics of those in whom the drugs failed. Characteristics like age, race/ethnicity, socioeconomic

status, and measures of obesity did not correlate with success or failure of treatment; but one measure that offered quite good predictive power was how well the participants responded to initial treatment with metformin. When TODAY began, study scientists checked to ensure that participants' diabetes had not already progressed to the point that metformin would be unable to provide adequate glucose control. To find out, they gave metformin for 2 to 6 months before the intervention formally began to all of those interested in joining the trial who otherwise met study requirements. Only those whose HbA1c was below 8 percent during this initial treatment with just metformin were able to participate in the full trial. The researchers of the current study found that for those accepted to participate, HbA1c values during this pre-study metformin-treatment test ranged from near normal (non-diabetic) for some participants to just under the 8 percent cut-off for others. In the new findings, researchers showed that these initial metformin treatment results were very good predictors of whether metformin or metformin plus rosiglitazone would succeed in the longer term: those participants whose HbA1c remained below 6.3 percent during the pre-study metformin test were much more likely to have well-controlled diabetes after 4 years than those whose HbA1c was higher during this initial period. An HbA1c of 6.3 percent is actually considered to be within the non-diabetic range (usually considered to be under 6.5 percent), and is below suggested treatment targets for adults (typically 7 percent), so health care providers may think their adolescent patients are doing very well even if their HbA1c is somewhat higher. However, this study shows that young people for whom metformin alone cannot keep HbA1c below 6.3 percent are at substantially increased risk for rapid type 2 diabetes progression, and should be carefully monitored in case more aggressive treatment is needed to keep blood glucose under control. Further research will be needed to determine whether additional treatment aimed at lowering HbA1c below 6.3 percent would help stave off disease progression in young people for whom metformin alone does not achieve that level of blood glucose control.

Zeitler P, Hirst K, Copeland KC, ... Wilfley D; for the TODAY Study Group. HbA1c after a short period of monotherapy with metformin identifies durable glycemic control among adolescents with type 2 diabetes. Diabetes Care 38: 2285-2292, 2015.

SCREENING FOR PREDIABETES

Improving on Methods for Diagnosing Prediabetes

in Africans: Research by NIDDK Intramural scientists has shown that it may be possible to improve on a screen for diabetes risk in Africa, a part of the world which is expected to see explosive growth of the disease in the coming decades. Although it was long associated with more prosperous countries, type 2 diabetes is increasingly becoming a problem in low- and middle-income countries, according to the International Diabetes Federation, which also estimates that in sub-Saharan Africa over two-thirds of people with diabetes are unaware they have the disease, the lowest rate of diagnosis anywhere in the world. Therefore, improving diagnosis is vitally important to bring life-saving diabetes treatment and prevention approaches to Africans who urgently need them. The hemoglobin A1c test (often referred to as “HbA1c”) was originally validated as a method for monitoring the effectiveness of diabetes treatment through the NIDDK’s landmark Diabetes Control and Complications Trial. In recent years, it has become accepted also as a means of diagnosing diabetes and prediabetes. Unlike other widely used tests for diagnosing type 2 diabetes and prediabetes, like the fasting plasma glucose and oral glucose tolerance test (OGTT), HbA1c has the advantage that it does not require previous fasting. However, HbA1c is not a perfect diagnostic test. For example, it does not correlate perfectly with average glucose levels: individual genetics have been shown to matter, particularly at near-normal glucose levels. Further, the test has not been validated for diagnosis of prediabetes or diabetes in people of completely African descent. NIDDK scientists reasoned that other tests that also do not require prior fasting—tests of total glycated albumin (GA) or of fructosamine—might potentially perform better than HbA1c for African patients. As a reference standard, they used OGTT, a test that is particularly time and labor intensive because it requires following a person’s response to an orally administered quantity of glucose over a period of hours, but also is most likely to be reliable, because it directly measures the effectiveness of the body’s response to glucose. In a study of 217 male and female African immigrants living in the United States, HbA1c tests correctly detected only half of the prediabetes cases identified by OGTT, while GA and fructosamine identified slightly fewer. However, the study showed that combining GA with HbA1c led to detection of significantly more of the prediabetes cases: 78 percent of those found through OGTT. On the minus side, combining HbA1c and GA tests

also increased the number of false positives (*i.e.*, reduced the specificity of the HbA1c test alone). However, it may be more practical to conduct OGTT tests only on individuals who turned up positive *via* a combined HbA1c/GA test than on a larger fraction of the at-risk population. It is important to remember that no test can determine exactly who will, in the fullness of time, eventually go on to develop type 2 diabetes. But quick and easy blood tests may prove to be a valuable tool for improving prevention, diagnosis, and treatment of type 2 diabetes, so that Africa may avoid some of the ravages of this disease.

Sumner AE, Duong MT, Aldana PC, ...Sacks DB. A1c combined with glycated albumin improves detection of prediabetes in Africans: The Africans in America Study. Diabetes Care 39: 271-277, 2016.

GENETICS OF TYPE 2 DIABETES

Genetic Trait in Pima Indians Linked to Increased Birth Weight and Elevated Risk for Type 2 Diabetes:

A genetic analysis in Pima Indians of the American Southwest has identified a rare mutation linked to elevated birth weight, that is later associated with higher risk of type 2 diabetes. Pima Indians have among the highest rates of diabetes in the world. To understand their unique genetic risk factors, and find ways to help alleviate this health disparity, NIDDK Intramural researchers examined the DNA sequences in and around a pair of genes thought to be involved in type 2 diabetes pathogenesis in 7,710 Pima study volunteers. They found that 3.3 percent of the participants had a previously uncharacterized variation in the gene *ABCC8*, which encodes a protein with a key role in regulating insulin secretion. The resulting genetic change—designated R1420H—was similar to known mutations that inactivate *ABCC8* and initially cause the pancreas to release more insulin than is needed but, for unknown reasons, later lead to a decline in insulin production, typically followed by type 2 diabetes.

A review of medical records showed that Pima babies who inherited a copy of the R1420H variant from one parent (and a normal copy of *ABCC8* from the other parent) tended to have a higher birth weight than siblings born with two normal copies of the gene. This is consistent with the expectation that these babies would be born with an excess of insulin, because insulin is a major growth factor during gestation. Further, the researchers found that as people with a copy of the R1420H version of *ABCC8* grow up, they had double

the risk of type 2 diabetes relative to Pima peers with two normal copies of *ABCC8*. This is despite having a lower body mass index (a measure of weight relative to height), on average, than other Pima adults at any given age; and Pima with a copy of R1420H develop the disease, on average, 7 years earlier than Pima with two normal copies of the gene. Why do genetic variants in *ABCC8* initially result in too much insulin, and eventually in too little? As with other mutations in *ABCC8* and its molecular partners, the reason is unclear and may be determined through further research. While rare, R1420H is common enough among the Pima that 1 in 3,600 babies born in the community would be expected to be born with two copies, and have no properly functioning *ABCC8*. One of the participants in the study had this genetic condition, and a review of medical records showed that he was born with severe hypoglycemia (low blood glucose), yet developed diabetes before the age of 4. These new findings could lead to tests to identify newborn Pima babies with two copies of the mutation, allowing early and effective intervention to improve the babies' health, and reduce their chances of death in infancy. Such a test would also help identify babies born with a single copy of R1420H, individuals who may benefit from keeping a careful eye on their blood glucose as they grow up, to allow timely intervention to prevent or treat type 2 diabetes. In a larger sense, also, the findings build on our knowledge of the genetics of type 2 diabetes. However, since R1420H is found in just 3.3 percent of the Pima population, this study does not resolve the question of what other genetic factors put this group at such a high risk for diabetes: those answers remain to be discovered.

Baier LJ, Muller YL, Remedi MS,...Bogardus C. *ABCC8* R1420H loss-of-function variant in a Southwest American Indian community: association with increased birth weight and doubled risk of type 2 diabetes. *Diabetes* 64: 4322-4332, 2015.

Variation in a Glucose Transporter Affects Response to the Type 2 Diabetes Drug, Metformin: New research indicates that a common variation in the gene encoding a protein that allows glucose (sugar) to move in and out of cells has a surprising impact on the effectiveness of the first-line anti-diabetes medication metformin.

Metformin is a very widely used, safe, and helpful treatment for type 2 diabetes, but it is more effective

in some people than in others, and scientists are trying to understand why. An international consortium of investigators looked at genomic variation in over 13,000 volunteers of varying ancestry who were taking metformin. They found that a common variation in the gene for a glucose transporter protein, GLUT2, had a significant impact on metformin effectiveness. (The gene encoding GLUT2 is known as *SCL2A2*.) Before treatment, people with two copies of a version of the gene (designated "C") typically had somewhat worse blood glucose control, as detected by higher levels of HbA1c, a marker for glucose levels. Yet, these individuals had slightly better (lower) HbA1c when taking a standard dose of metformin than did people with two copies of the other version ("T") of the GLUT2-encoding gene. This effect was most pronounced in people who were obese, but was also seen in those who were not. People with one copy of each version had an intermediate response to metformin. GLUT2 allows glucose to move passively in and out of cells in the liver, an organ with a critical role in regulating blood glucose levels. The GLUT2 that is produced by the C and T versions of the gene is the same, equally capable of allowing glucose movement. However, the researchers found that liver cells with the C version make less GLUT2 than liver cells with the T version. This suggests that in the absence of metformin, individuals with type 2 diabetes and the C version are at a disadvantage compared to those with the T version when it comes to regulating blood glucose levels, but that metformin treatment overcomes and even slightly reverses this effect. Metformin still works in people with two copies of the T version of the gene, but more of the drug—or an additional medication—would be needed to achieve the same degree of HbA1c reduction.

This discovery has broad applicability, because the C and T versions of the gene are both common in a wide variety of racial/ethnic groups, albeit to differing degrees. For example, about 70 percent of African Americans have at least one copy of C, while 24 percent of Latinos do. With further research, tests to reveal a patient's GLUT2 gene version could one day help further precision medicine by allowing health care providers to tailor metformin dosage for that individual, so that he or she takes neither more nor less of the medication than needed.

Zhou K, Yee SW, Seiser EL,...Pearson ER. Variation in the glucose transporter gene *SLC2A2* is associated with glycemic response to metformin. *Nat Genet* 48: 1055-1059, 2016.

Diabetes Portal Adds Data, More Powerful Search Tools: Detailed Content Now Available to Everyone



The AMP Type 2 Diabetes Knowledge Portal (www.type2diabetesgenetics.org/) online library and discovery engine has greatly expanded data and search capabilities to accelerate the pace of scientific advancement. Simplified, customizable navigation of aggregated data from more than 100,000 DNA samples from research supported by the NIH and other institutions facilitates new understanding of diabetes by increasing users' ability to share and evaluate content.

A product of the NIH's Accelerating Medicines Partnership for type 2 diabetes (AMP T2D), the portal—which opened in 2015—enables user-friendly exploration of international networks of human genetic information linked to type 2 diabetes. At present, researchers and the public can search for information by gene, genetic variant, and region; access summaries of genetic variants; and run customized genetic analyses using versatile tools. Personal identifying information will remain confidential.

Anyone can now query detailed data from the portal. Previously, only approved researchers could access that content, while others could view aggregate results. A Google account is all that is needed to use the portal, which is also available in Spanish. Because the power of the portal depends on community participation, people are encouraged to submit data, comments, and other materials. Administrators also continue to expand the network to include more national and international content, such as the recent addition of data from European collaborators. Funding for the portal is provided through grants from the NIDDK and the Foundation for the National Institutes of Health. Additional support is provided by the Carlos Slim Foundation, Mexico City. The awards are part of a larger partnership of academic investigators, the NIH, and five pharmaceutical companies. AMP T2D was conceived to translate findings on genetic risk factors in type 2 diabetes into valid targets for new therapies or treatments and provide insights into the pathogenesis and heterogeneity of diabetes.

MOLECULAR UNDERPINNINGS OF GLUCOSE CONTROL AND INSULIN RESISTANCE

A Molecular Signal That May Lead to Insulin

Resistance and Type 2 Diabetes: New research in mice has identified a compound that triggers increased uptake of “fatty acids”—a category of fat molecules—by blood vessels and muscle cells, and that may thereby contribute to insulin resistance. Experimental evidence suggests that in type 2 diabetes, accumulation of fatty acids in muscle cells may play an important role in reducing the cells’ capacity to respond to insulin by absorbing glucose from the blood; but the mechanism by which fatty acids become concentrated in muscle cells to have this effect has remained unclear. Researchers considered the possibility that a protein called PGC-1 α may be involved: among its many roles in the body, it is responsible for increasing the ability of muscle cells to utilize fatty acids by signaling the cells to make more of the enzymes that break them down for fuel. The scientists reasoned that PGC-1 α may also trigger secretion of a chemical signal to adjacent blood vessels that acts as a request to supply the muscle cells with more fatty acids. To test this idea, the scientists grew muscle cells that make extra PGC-1 α , and then collected the liquid they had grown in—which would contain anything they secreted. When the scientists then transferred this liquid to cultures of blood vessel cells, they found that it caused a marked increase in uptake of fatty acids.

By comparing substances secreted by normal muscle cells with those from cells with elevated levels of PGC-1 α , they were able to single out a small molecule designated “3-HIB” as the likely signaling molecule. PGC-1 α causes muscle cells to increase the breakdown of valine, an essential amino-acid building block of protein, into 3-HIB, among other compounds; and some of the resulting 3-HIB is then secreted. The researchers found that simply adding 3-HIB to cultured blood vessel cells induced them to increase their uptake of fatty acids. Further, they found that mice given 3-HIB in their drinking water accumulated more fatty acids in their muscle cells than mice not consuming the compound, and also became insulin resistant. (The experiments reported were performed with male mice; female mice may or may not have responded similarly.) Interestingly, previous research has shown that elevated blood levels of valine (as well as certain other amino acids) are associated with a higher risk of developing

type 2 diabetes in humans, but the reasons for the correlation remained mysterious. This new study suggests one possible explanation: that excess signaling by 3-HIB, a breakdown product of valine, may contribute to the development of type 2 diabetes. If confirmed, a therapeutic agent that interferes with valine breakdown or 3-HIB signaling may one day help treat or prevent type 2 diabetes.

Jang C, Oh SF, Wada S,...Arany Z. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. Nat Med 22: 421-426, 2016.

Newly Identified Molecule Modulates Glucose Release by the Liver:

Scientists have discovered a molecule that shows potential as a therapeutic target in people with type 2 diabetes and metabolic syndrome. The molecule, a protein called asprosin, was identified by studying people with a congenital condition that, among other things, causes them to have partial lipodystrophy, or lack of fat tissue in certain areas of the body. Lipodystrophy is often accompanied by insulin resistance and increased insulin levels (a response to insulin resistance), and thus a high risk of developing type 2 diabetes, as well. However, the researchers found two patients who, surprisingly, were not insulin resistant, had lower than normal insulin levels, and normal blood glucose (sugar) levels. By conducting genetic analyses in these two people and examining published scientific reports describing this condition in several other people, the researchers discovered that they all had mutations in a gene coding for a protein called fibrillin. These mutations caused cells to make and secrete much less asprosin, which is derived from fibrillin.

But what does asprosin do? To find out, the researchers conducted experiments in healthy male mice and found that asprosin levels in the bloodstream dropped with the onset of eating. In other experiments, analyzing both mice and humans, they showed that asprosin levels rose in response to fasting. Further experiments in mice and mouse cells revealed that asprosin targets cells in the liver that store and release glucose to help regulate blood glucose levels—a critical metabolic function. When asprosin interacted with these cells, glucose was released into the bloodstream. At the same time, insulin levels rose quickly to counteract the rise in glucose levels, and appeared to help suppress asprosin’s stimulation of glucose release from the liver

cells. These results suggest that asprosin may play a key role in regulating blood glucose in response to food intake.

Excess release of glucose from an insulin-resistant liver is a problem in type 2 diabetes. The researchers observed that asprosin levels were much higher than normal in both male humans and male mice with insulin resistance and elevated insulin levels, suggesting that asprosin may be involved in this metabolic dysfunction. To see if targeting asprosin might be of therapeutic use, the scientists conducted several experiments, including “blocking” its activity in insulin-resistant, obese male mice by using an antibody directed against the molecule. Encouragingly, injection of a single dose of the antibody caused both asprosin and insulin levels to drop for several hours in the mice, while their blood glucose levels remained stable. In combination with other experimental results, these findings suggest that blocking asprosin leads to a reduced glucose burden in the blood that can be regulated with less insulin. While additional research needs to be pursued in both animal models and people, including investigating whether asprosin activity is the same in females and males, these and other experimental results suggest that artificially lowering asprosin levels may be a new approach to help improve conditions and diseases rooted in insulin resistance, such as metabolic syndrome and type 2 diabetes.

Romere C, Duerrschmid C, Bournat J,...Chopra AR. Asprosin, a fasting-induced glucogenic protein hormone. *Cell* 165: 566-579, 2016.

METABOLIC REGULATORS OF HEALTH AND DISEASE

Brain Cells That Control the Body’s Response to Heat: New research in mice identifies a subset of cells in a specific region of the brain that controls how mammals likely respond to heat. It is critical that our bodies are able to maintain a stable temperature, because sustained periods when core body temperature is too hot or too cold can be dangerous. Mammals have developed various ways to combat temperature fluctuations, such as sweating to cool down or shivering to warm up. In addition to these involuntary responses, mammals also change their behavior, like burrowing for warmth or seeking cool locations. Temperature is sensed

by the skin, and this information is relayed to the brain where it is translated into physiological and behavioral responses. Past studies have pointed to a specific region of the brain called the preoptic area, or POA, as being strongly associated with temperature regulation. Previous experiments have shown that heat stimulation of the POA generated substantial regulatory responses to cool the body, while damaging this region eliminated this response. However, the specific cells in the POA that receive temperature information and how they coordinate the appropriate response are poorly understood.

To uncover these details, researchers first sought to identify genes that are “turned on” when both male and female mice were exposed to heat. The levels of gene activity were then used as markers to reveal the brain cells that were activated when the mice were exposed to heat (“warm-sensitive neurons”). Through a series of experiments, the scientists discovered the identified brain cells are specifically and rapidly activated by warm temperatures that trigger temperature-regulating reactions in the mice, indicating that these cells are sufficient to regulate the complex response to heat. The team used a genetic technique to insert a light-responsive protein into the specialized cells to control their activity. This allowed the researchers to turn on the warm-sensitive cells with light. Activation of the cells triggered a rapid decline in core body temperature of the mice. The scientists observed an increase in tail temperature to dissipate heat, and a decrease in the temperature of brown adipose (fat) tissue, which generates heat. In addition to the involuntary physiological responses, experimental activation of the cells also induced behavioral changes. The mice sought out cooler temperatures and decreased nesting activity. Using imaging techniques, the researchers were also able to visualize the heat-sensitive cells making connections with areas of the brain that regulate physiological and behavioral responses to heat. No differences were observed between male and female mice.

These results provide new insights into our understanding of how body temperature is regulated by the brain. The identification of neurons that act as mission control centers to receive environmental information and disseminate messages to alter body temperature in response is a breakthrough and a potential target for therapeutic manipulation. More

research is needed to determine if analogous cold-sensing cells exist.

Tan CL, Cooke EK, Leib DE,...Knight ZA. Warm-sensitive neurons that control body temperature. *Cell* 167: 47-59, 2016.

(Information adapted from original article by Dr. Tianna Hicklin, published on October 4, 2016 in NIH Research Matters).

Identification of Exercise Molecule That Promotes Physical Endurance: Researchers discovered that musclin, a protein released by skeletal muscle in response to physical activity, enhances exercise capacity in mice. The benefits of exercise are numerous and varied, but how these benefits are achieved at a molecular level remains poorly understood. Skeletal muscle releases proteins, called myokines, and understanding the activity of myokines could lead to new therapies that provide the benefits of exercise. In this study, scientists focused on the relationship between physical activity and a previously discovered myokine named musclin. To determine whether or not musclin was associated with exercise, they looked at the levels of musclin in a group of mice that exercised on a treadmill daily in comparison to a sedentary group. In addition to finding increased levels of musclin in the muscle of the active mice, musclin was increased in the mice's blood, suggesting that it could have both local and systemic effects.

To identify the effects of exercise-induced musclin, the scientists genetically engineered mice to lack musclin. Compared to mice with musclin, the mice without the protein showed less physical endurance; they tolerated less time, distance, and overall workload on the treadmill. When these mice were given musclin through an infusion, they increased their exercise to normal levels. This demonstrated that musclin was responsible for the differences in physical endurance, and that musclin has potential as a therapeutic to increase exercise tolerance, making it easier for people to exercise. Disruption of musclin also altered the oxygen consumption of the mice during exercise and the size of mitochondria—the energy powerhouses of the cell—in the mice's muscles, revealing how musclin affects the production of energy in the muscles.

This study showed that levels of musclin are increased in mouse skeletal muscle in response to exercise, and that loss of musclin decreases exercise endurance

and oxygen consumption. Importantly, the scientists showed that human muscle cells in the laboratory have musclin, but additional research will be needed to establish whether human musclin acts in the same way as the mouse version. By finding a link between musclin and physical activity, this study identifies musclin as a potential therapeutic to help people receive the benefits of exercise.

Subbotina E, Sierra A, Zhu Z,...Zingman LV. Musclin is an activity-stimulated myokine that enhances physical endurance. *Proc Natl Acad Sci USA* 112: 16042-16047, 2015.

CYSTIC FIBROSIS RESEARCH

Understanding Differences Between Mouse and Human Cystic Fibrosis Suggests a Potential Way To Reduce Infections: New research has shown why mice that have the mutation that causes cystic fibrosis (CF) avoid the repeated, serious bacterial lung infections that gradually cause severe lung damage in people with the disease; if a medicinal approach can be found that safely makes the human CF lung environment more like that of mice, this may help people with CF avoid some of the disease's most dangerous and debilitating effects.

Soon after the discovery that a mutation inactivating the CFTR protein is the cause of cystic fibrosis, scientists created mice with the same mutation in hopes of better understanding the human disease, and to more easily test promising new approaches to CF treatment. To their surprise, however, mice lacking functional CFTR were not nearly as sick as people without it are. Among other differences, the mice are much more resistant to bacterial infections of their lungs, and have mucous in the lungs that is not quite as thick as in human CF. Since then, researchers developed other animal models that more closely resemble the human form of the disease—such as CF pigs and ferrets. But a nagging question remained: what makes CF mice uniquely resistant to infection? A variety of explanations have been suggested. One recently offered hypothesis has to do with acidity—the fluid lining the lungs (“airway surface liquid”) is more acidic in people with CF than in people without the disease. Recent findings suggest this acidification makes it harder for the lungs to fend off bacteria, while simultaneously contributing to the viscosity and

stickiness of airway surface liquid in CF. Bolstering this hypothesis, airway surface liquid also becomes acidic in pigs with CF—but not in CF mice.

New research indicates that acidity does indeed account for much of the difference in infection susceptibility between humans and mice with CF, and identifies the specific protein responsible for acidification of airway surface liquid in people with the disease. The researchers noted that acidity could result either from an excessive transport of acid-causing protons into the airway surface liquid, or from a failure of CF lungs to pump out enough acid-neutralizing ions to counteract protons that are normally there. Indeed, fully functioning CFTR is needed to move bicarbonate, an acid-neutralizing molecule, out of airway cells and into the airway surface liquid. Because CF mice have as much trouble transporting bicarbonate as do humans with the disease, the researchers reasoned that the mouse advantage likely stems from having fewer acidifying protons to neutralize in their airway surface liquid. A careful comparison of mouse and human airway cells suggested that this was, in fact, the case, and implicated a protein called ATP12A that moves protons out of airway cells into the surface liquid, in exchange for potassium ions that it moves into the cells. Mouse airway cells have almost no ATP12A on the outer surface of their airway cells, while those of people and pigs have quite a bit of the protein. In an experiment with cultured human and pig CF airway cells, applying ouabain, a chemical that inhibits proton transport by ATP12A, prevented the cells from acidifying the liquid they were growing in, and made the cultures significantly more resistant to infection, while also reducing the viscosity of the liquid. They achieved similar results by removing potassium ions from the culture liquid, which also prevents ATP12A from transporting protons. In contrast, when they experimentally introduced ATP12A into the airways of CF mice, they found that these mice were much more susceptible to lung infection.

This study suggests that preventing airway acidification could be a new approach to CF therapy. The experimental strategies used in the study, eliminating potassium, or administering ouabain, are not likely to be feasible therapeutically, because potassium is essential for normal function of critical

tissues, and ouabain can be quite toxic. However, less toxic ATP12A inhibitors may be identified; and another potential treatment approach might be to neutralize acid in the airway surface liquid using a solution of bicarbonate or other non-toxic buffer. Future research is needed to determine whether these sorts of approaches can safely and effectively improve infection resistance and/or thin the viscous airway surface liquid of CF patients.

Shah VS, Meyerholz DK, Tang XX, ...Welsh MJ. Airway acidification initiates host defense abnormalities in cystic fibrosis mice. Science 351: 503-507, 2016.

SCREENING FOR LYSOSOMAL STORAGE DISEASES

Improving Newborn Screening for Lysosomal Storage Diseases: Scientists developed a new method to screen newborns for six lysosomal storage diseases simultaneously. Lysosomal storage diseases are a group of inherited metabolic conditions in which certain molecules accumulate in harmful amounts in the body's cells and tissues. Each of these diseases is caused by the lack or deficiency of a different protein (enzyme) responsible for degrading waste molecules in lysosomes, which are structures found in cells throughout the body where critical cellular recycling processes occur. The symptoms of the various lysosomal storage diseases vary significantly, and often are not immediately apparent at birth, but each involves the toxic buildup of metabolites that can damage multiple organs in the body. These diseases can cause significant pain; neurological, heart, liver, or kidney problems; and premature death. Because several lysosomal storage diseases are treatable, and early treatment can lead to better outcomes, there is interest in increasing current newborn screening to include treatable lysosomal storage diseases. In many states, newborns are screened for specific disorders using dried blood spots on screening cards. Previous research demonstrated that lysosomal enzymes (such as those missing in this cluster of diseases) retain their activities when the dried blood spots are rehydrated, indicating that screening assays based on direct enzymatic activity could be possible.

In this study, researchers developed and evaluated a new test designed to efficiently and simultaneously screen newborns for Pompe, Mucopolysaccharidosis-I, Fabry, Gaucher, Niemann Pick-A/B, and Krabbe lysosomal storage disorders. In this pilot study, they performed the test on around 43,000 de-identified newborn dried blood spots. Because the samples were de-identified, the researchers could not determine whether their test had successfully pinpointed all cases of these diseases from among the samples. However, by sequencing the key gene from each apparent case of

a lysosomal storage disorder the test did identify, the researchers were able to infer what appeared to be a reasonable, low rate of false-positives, that would presumably be corrected by further testing. This technical achievement is a proof of principle, showing that this type of approach may one day soon lead to improved and expanded newborn screening for lysosomal storage disorders.

Elliott S, Buroker N, Courmoyer JJ,...Scott CR. Pilot study of newborn screening for six lysosomal storage diseases using tandem mass spectrometry. Mol Genet Metab 118: 304-309, 2016.

Islet Transplantation—A Promising Treatment for Difficult-to-treat Type 1 Diabetes

Decades of research, funded by NIDDK and others, is bringing a potential life-changing treatment—*islet transplantation*—closer to a reality for people with type 1 diabetes. People with type 1 diabetes are unable to take in glucose (sugar) from the blood and use it to fuel their bodies. Without glucose, the cells of the body starve. In 1921, scientists identified the factor that directed glucose from the blood into cells—the hormone *insulin*—and changed the course of treatment for the disease. Insulin is produced by β (beta) cells, which inhabit the pancreas in clusters known as *islets*. It is now known that, in people with type 1 diabetes, the body mounts a misguided immune attack against its own β cells. Some or all of the β cells are destroyed, leaving the body without the ability to produce insulin.

A person with type 1 diabetes can take insulin, by injections or with an insulin pump, which is a life-saving treatment. To estimate how much insulin their bodies may need, people with the disease must closely monitor their diet, exercise, and daily routine. Despite careful management of diabetes, it is difficult to mimic the exquisite blood glucose control of the pancreas. While taking insulin treats excess glucose in the blood (*hyperglycemia*), too much insulin can lead to a lack of glucose (*hypoglycemia*) in the brain and dangerous situations including coma and death. This absence of normal glucose control leads to diabetic complications.

Even with today's improved, long-lasting formulations of insulin, this treatment is burdensome and does not work for everyone. Despite vigilant insulin administration, people with difficult-to-control diabetes (also called "*brittle diabetes*") may have episodes of severe hypoglycemia with memory loss, confusion, altered or irrational behavior, difficulty in

awakening, seizures, or loss of consciousness. Such episodes may make driving or caring for young children unsafe. Repeated episodes can lead to "*hypoglycemia unawareness*," in which a person does not realize that he or she has dangerously low blood glucose levels, and thus does not recognize the situation and/or is unable to self-administer treatment. It is critical, therefore, to work toward improved treatments, prevention strategies, and possible cures for type 1 diabetes.

Researchers believe that *islet transplantation* could be a potential alternate treatment, especially for people with brittle diabetes. In current *islet transplantation* procedures, the islets are removed from the other cells of a deceased organ donor's pancreas using specialized enzymes. The islets are purified, processed to maintain their viability and improve engraftment, and counted in a laboratory to ensure that there is a sufficient number for the transplant. After X-rays and ultrasound guide placement of a thin, flexible tube called a catheter through a small incision in the upper abdomen, the surgeon infuses the islets slowly through the catheter into the portal vein of the liver. Once implanted, the islets engraft into the liver tissue and begin to make and release insulin. Full islet function and new blood vessel growth from the new islets take time. Transplant recipients usually take insulin injections until the islets are fully functional. Medications suppressing the immune system are needed for *islet transplantation*, and must be continued as long as the transplanted islets function to prevent rejection of the transplant. Because of the side effects of these medications, *islet transplantation* is only considered appropriate for people with incapacitating hypoglycemia despite therapy from physicians skilled in treating type 1 diabetes or for

STORY OF DISCOVERY

those with kidney transplants who already require immunosuppression to preserve the function of the transplanted kidney. Currently, islet transplantation is an experimental therapy, one that can only occur within a clinical trial. For this procedure to transition to a therapy that can be conducted outside of clinical trials, human islets would need to be approved as a cellular biological product by the U.S. Food and Drug Administration (FDA). A Biologics License Application (BLA) will soon be submitted to the FDA, representing decades of research progress to develop innovative solutions to significant challenges.

Transplantation To Restore Insulin Production—From Whole Pancreas to Islets

The idea of transplanting insulin-producing tissue into a person with type 1 diabetes is not a new one. In the late 19th century, scientists discovered that removal of the pancreas caused a dog to develop diabetes, and that transplantation of healthy pancreatic fragments into a diabetic dog could prevent the mortality associated with removing the pancreas. This seminal finding launched the field of pancreas transplantation, and the following year an English surgeon transplanted pancreatic fragments from sheep into a 15-year old boy with severe diabetes. The boy demonstrated temporary improvement of his diabetes, but died several days after the transplant. Doctors have since improved whole pancreas transplantation from human donors, but the procedure involves invasive surgery and, therefore, is best when it can be performed at the same time as another organ transplant, such as a simultaneous kidney and pancreas transplant.

In 1972, scientists achieved the first successful islet transplantation in laboratory rats, reversing their chemically induced diabetes. This success invigorated the field and described several techniques for harvesting, purifying, and transplanting the islets that laid the path toward human clinical trials. However, scientists had difficulty repeating the procedure

in larger animal models due to differences in the structure, size, and shape of rodent islets compared to large mammalian islets. In addition, researchers had difficulty in extracting and purifying sufficient numbers of human islets for transplantation. A major advance toward improving approaches to isolate human islets occurred in 1988. Researchers developed an automated method that involved minimal traumatic action on the islets, continuous digestion of the pancreas to release islets, removal of islets to avoid over-digestion, and minimal human intervention in the digestion process. With this new method, islets could be purified more easily, in larger numbers, and in better quality. This advance renewed interest in the possibility of human islet transplantation as a treatment for type 1 diabetes.

Challenges to Islet Transplantation

Another challenge to islet transplantation and to any organ transplantation is the potential rejection of the islets as “foreign” tissue. Scientists think it is likely that many early attempts at islet transplantation—and transplantation of other organs—failed because the recipient’s immune system attacked the transplanted tissue. In addition to the immune system attack on the transplanted tissue as foreign, the continued autoimmune attack on β cells, the same misguided attack that destroyed a person with type 1 diabetes’s own β cells, can reduce the success of islet transplantation. The field of transplantation was propelled forward with the development of immunosuppressive drugs, which dampen the transplant recipient’s immune system.

In 1989, NIDDK-supported researchers combined the new method for isolating human islets with advances in immunosuppression to report the first human islet transplantation that resulted in insulin independence—being free from the need for insulin injections or a pump, because the body was making its own insulin. Ten days after the transplant, the recipient stopped taking insulin

STORY OF DISCOVERY

and maintained acceptable blood glucose levels for 12 days. After this period, her glucose levels rose, and she needed to take insulin again. Continued improvements in immunosuppressive medications and procedures to harvest, isolate, purify, and preserve islets led to additional reports of successful islet transplantations with insulin independence for longer periods, even over a year post-transplant, although results varied from patient to patient and study to study.

Immunosuppressive drugs became standard therapy for transplants, but with serious associated risks. Their use can lead to significant side effects, including increased susceptibility to bacterial and viral infections, fatigue, decreased kidney function, mouth sores, and gastrointestinal problems. The long-term side effects are not fully known, and taking immunosuppressive medications also increases the risk of developing certain cancers. These immunosuppressants are thought to affect also the long-term viability of the transplanted islets, as studies suggest that they are toxic to the islets over time. Thus, researchers were searching for ways to enhance the standard therapy using immunosuppressives to improve islet viability and transplantation outcomes.

Significant progress toward that goal was made in 2000, when researchers in Edmonton, Canada, utilized a new protocol that tested a novel combination of immunosuppressive drugs. In a small study, seven people with type 1 diabetes achieved normal blood glucose levels following islet transplantation using this new "Edmonton protocol." Each patient received a large number of islets in two or three transplants. Progress in methods for isolating and storing islets from donor pancreata prior to transplantation also added to the success of the trial. However, while patients maintained normal blood glucose levels for a period after the transplant, the islets tended to lose their insulin-producing function over time. In addition, it remained to be demonstrated

whether Edmonton's success could be replicated at other sites around the world in a standardized way.

Standardizing Islet Transplantation

The Immune Tolerance Network, an international consortium led by the National Institute of Allergy and Infectious Diseases (NIAID) in collaboration with the NIDDK and JDRF (formerly Juvenile Diabetes Research Foundation International), took on the challenge to replicate the Edmonton protocol in a multi-center trial. In the first multi-center trial of islet cell transplantation, from 2001 to 2006, nine sites in North America and Europe successfully replicated the Edmonton protocol in 36 people with type 1 diabetes. One year after transplant, 44 percent of the participants achieved insulin independence with good glycemic control, and another 28 percent, although still requiring some insulin, had partial graft function that completely protected them from severe hypoglycemic episodes. Protection from severe hypoglycemic episodes is an important outcome, as hypoglycemic episodes endanger the lives of people with type 1 diabetes, and fear of hypoglycemia is one reason people with the disease do not achieve the recommended blood glucose levels. At the 5-year evaluation after their final transplant, 17 percent were still insulin-independent.

Although insulin independence declined over time in the study participants, this important study demonstrated that the success of the Edmonton trial could be replicated in a standardized way at other locations. Refinements in techniques for preserving the pancreas and preparing the islets, as well as initiating immunotherapy prior to transplantation to improve islet engraftment, led to high rates of insulin independence in another study of eight single-donor islet transplants. These studies laid the groundwork for a large, international, multi-center network to study and refine islet transplantation technology and provide data toward submission to FDA of an application for islets as a biological product.

STORY OF DISCOVERY

The Clinical Islet Transplantation Consortium: Working Towards FDA Approval of Islet Transplantation

As knowledge of islet cell biology and the processes associated with transplantation and immune rejection increased, and pre-clinical studies evaluating new approaches to immunomodulation in conjunction with islet transplantation in animal models progressed, a means was needed by which to study these new approaches rigorously. In 2004, NIDDK and NIAID established the Clinical Islet Transplantation (CIT) Consortium to provide a well-coordinated, collaborative approach to find islet transplantation methods that have higher success rates and fewer risks. To find participants that would benefit the most from this treatment, CIT researchers screened over 8,000, enrolled about 450, and transplanted over 125 participants. CIT has conducted eight clinical trials, with associated immunologic, metabolic, and mechanistic studies, of islet transplantation in individuals with difficult-to-control type 1 diabetes despite intensive medical management.

One of these trials, a phase III study of islet transplantation, enrolled 48 people with type 1 diabetes, impaired awareness of hypoglycemia, and frequent, severe hypoglycemic events despite expert care. At 2 years after transplantation, more than 70 percent of participants demonstrated excellent blood glucose control (with an overall average HbA1c level less than 6 percent), and freedom from severe hypoglycemic events, with restored hypoglycemia awareness. These findings indicated that islet transplantation is an effective treatment for people who have severe hypoglycemic events even with the best medical care. Though these results are impressive, the procedure had significant side effects. Although less toxic, the immunosuppressive drugs still had substantial risks and were demonstrated to decrease kidney function as seen in other transplantation trials. Therefore, this procedure should

only be undertaken by people whose type 1 diabetes cannot be controlled by other means and for whom hypoglycemic episodes are life-threatening.

Manufactured islets are not currently approved by the FDA, meaning that islet transplantations cannot be conducted outside of clinical trials. Much like a new drug, islets produced for islet transplantation need to be produced at a high quality consistently. This CIT trial utilized a standardized protocol for generation of islets for transplantation across multiple sites, so that the results could be used as the basis to apply for FDA licensure of islets as a biologic product. Licensure would ensure the purity, potency, and safety of a standard islet product. Once the islet product is FDA-approved, it could transition islet transplantation from an experimental treatment to one that could be performed in regular practice, outside of clinical trials. At that point, the procedure could be covered by third-party insurers. To continue to advance this field as rapidly as possible, scientists need access to information on every islet transplant that takes place, not just those in their local facilities. Thus, the NIDDK created the Clinical Islet Transplantation Registry in 2000 to collect data on islet transplantations for use by the scientific community and the public. By collecting and analyzing these data, the Registry is helping to define the overall risks and benefits of islet transplantation as a treatment option for people with difficult-to-control type 1 diabetes, which is informing future research efforts.

Looking to the Future

Additional efforts are under way to tackle several of the ongoing challenges to long-term success in islet transplantation. A major challenge is the lack of islets available for transplant. Currently, only donor pancreata are used for islet transplantation; a limited number of donor organs are available in general, and they need to match the recipient on several criteria. Additionally, these organs are only available for islet transplantation if the organ has been deemed

STORY OF DISCOVERY

unsuitable for whole pancreas transplantation. Compounding this problem is that studies have demonstrated that large numbers of islets, often from multiple donors, are required to improve the likelihood of success. Thus, it is imperative to identify alternate islet sources. Toward this goal, researchers are exploring ways to generate human islets in the laboratory, and recent advances in this field, including the creation of stem cell-derived β cells from people with type 1 diabetes reported in this chapter, are accelerating progress. The NIDDK's newly established Human Islet Research Network is conducting basic research studies to pursue innovative strategies to protect and replace β cells in people with diabetes, including developing approaches to grow human islets in the laboratory (see Feature in this chapter). Scientists are also developing ways to protect transplanted islets to improve their longevity and function and to reduce the need for the toxic immunosuppressive medications. Researchers are evaluating new immunosuppressive medications, new combinations of immunosuppressive medications, and non-immunosuppressive medications, as well as ways to induce tolerance in the immune system to protect the transplanted islets. Another strategy being studied to protect newly transplanted islets from immune system attacks is to encapsulate them in a special material; promising results in this area are also described in this

chapter. Advances in these fields will be critical for developing a durable cellular therapy for more people with type 1 diabetes.

Researchers have shown that individuals with difficult-to-control type 1 diabetes who receive transplanted islets can have greatly reduced episodes of hypoglycemia, and some remain free of insulin injections for extended periods of time. Islet transplantation, at its current state of development, has been shown to be an effective therapy for this population, a small subset of people with type 1 diabetes. In addition to efforts to improve islet transplantation and other methods to replace β cells, improved technologies for managing diabetes are also being developed, and it is hoped that the population with difficult-to-control diabetes will decrease as these technologies emerge. To develop all possible options to treat type 1 diabetes, researchers continue to pursue islet transplantation. A continued, multi-pronged research approach with pivotal trials of state-of-the-art methods of islet transplantation in humans, as well as basic and pre-clinical efforts, will advance the procedure. As techniques improve and islet transplantation becomes safer and with fewer side effects, the benefits of this treatment may outweigh the risks, making it an option for a larger number of people with type 1 diabetes.

Dr. Alan R. Saltiel— Out of Balance: Obesity, Inflammation, and Diabetes

Dr. Alan R. Saltiel is a professor at the University of California, San Diego School of Medicine, where he is the Director of the school's new Institute for Diabetes and Metabolic Health. Dr. Saltiel is an internationally recognized expert on the hormone insulin and its role in obesity, diabetes, and cellular signaling. He has published more than 280 original papers, has developed drugs for diabetes and cancer, and holds 18 patents. Dr. Saltiel has received numerous awards, including the Rosalyn Yalow Research and Development Award from the American Diabetes Association, the Hirsch Award, and both the John J. Abel Award in Pharmacology and the Louis S. Goodman and Alfred Gilman Award in Receptor Pharmacology from the American Society for Pharmacology and Experimental Therapeutics. He is a member of the National Academy of Medicine (formerly the Institute of Medicine) and a fellow of the American Association for the Advancement of Science. Dr. Saltiel was appointed to the National Diabetes and Digestive and Kidney Diseases Advisory Council in January 2015.

The seriousness of the obesity epidemic has been well-established: approximately one-third of U.S. adults live with obesity, and obesity increases the risk for type 2 diabetes, kidney disease, and many other devastating diseases and conditions. As researchers seek new ways to treat and prevent obesity, one question is: how, on the cellular level, does obesity harm the body such that excess fat increases risk of health problems? Dr. Saltiel's research on the links between obesity, inflammation, and diabetes strives to answer this question.

At the National Diabetes and Digestive and Kidney Diseases Advisory Council meeting in May 2016, Dr. Saltiel shared some of his research group's findings about how obesity causes an inflammatory response that has widespread effects on metabolic health. This research supports a model of metabolic health and disease in which the body views obesity as a stress that it attempts to remedy by triggering inflammation. That inflammation can lead to a shift in the metabolic "set point" that maintains weight and blood glucose (sugar) at heightened levels, but ultimately, the continued stress of obesity and inflammation can lead to insulin resistance and type 2 diabetes.

Warning Signal: Inflammation and Fat (Adipose) Tissue

Inflammation has been linked to obesity for decades, since inflammatory signaling proteins and inflammation-associated immune cells were found in fat (adipose) tissue. In the mid-2000s, these immune cells were still being characterized, and their role in obesity was unclear. Dr. Saltiel's research team discovered that in lean mice, adipose tissue was rich in a type of immune cell called an M2 macrophage. These M2 cells were known to function in tissue repair and to perform anti-inflammatory functions. If mice were fed a high-fat diet, however, the fat cells became stressed, releasing signaling proteins that recruited pro-inflammatory M1 macrophages as well. The M1 cells eventually overcame the anti-inflammatory activity of the M2 cells, an inflammatory state set in, and the fat cells became resistant to the actions of the hormone insulin. This insight was an important advance in understanding the link between inflammation and

SCIENTIFIC PRESENTATION

diabetes, because insulin resistance is a precursor to, and also a feature of, type 2 diabetes.

One observation that intrigued Dr. Saltiel was that even though fat cells would become insulin resistant when mice were given a high-fat diet, the cells would still continue to store energy—building up body fat from calories. Insulin promotes fat storage in adipose tissue (in addition to its role in blood glucose control). But were signals other than insulin contributing to fat storage? The answer, Dr. Saltiel's group found, was yes. Mice fed a high-fat diet not only showed hallmarks of low-grade inflammation but also increased activation of a signaling protein called IKK ϵ . To elucidate IKK ϵ 's role in inflammation and obesity, Dr. Saltiel's group genetically engineered mice to lack the IKK ϵ gene. When those mice were put on a high-fat diet, they gained less weight, had much less inflammation, and had much better insulin responses than did normal mice put on the same diet. They also increased their calorie burning (energy expenditure). The researchers concluded that IKK ϵ was, indeed, responsible for inflammation in response to a high-fat diet and also for downstream effects on glucose and fat storage. This effect also seemed to be related to a change in calories burned: the mice lacking IKK ϵ had increased body temperature and an increase in oxygen consumption, both indicators of increased energy expenditure.

Breaking the Cycle: Treating Obesity by Preventing Inflammation

To shed more light on these findings and investigate IKK ϵ signaling as a potential therapeutic target, the researchers sought an inhibitor of IKK ϵ that could be tested in mice. Dr. Saltiel and his colleagues performed an extensive test of over 150,000 chemical compounds for their ability to inhibit IKK ϵ , and one “hit” was a drug called amlexanox. Further investigation into the scientific literature found that amlexanox had been developed in 1985 and had been used in Japan

to treat asthma, though how it worked was not fully determined. The drug also had very few reported adverse side effects.

To determine if inhibiting IKK ϵ would have an effect on obesity, Dr. Saltiel's group gave amlexanox to mice on a high-fat diet. The drug protected the mice from the associated weight gain. Amlexanox also caused already obese mice on a high-fat diet to lose weight, and if the amlexanox was withdrawn, the mice regained the weight. Thus, amlexanox could both protect against and reverse the effects of a high-fat diet, promoting weight loss. Additionally, amlexanox treatment helped restore other signs of metabolic health: compared to mice not receiving the drug, mice receiving amlexanox had improved glucose tolerance, better insulin sensitivity, and reduced adipose tissue inflammation on a high-fat diet.

But how did amlexanox cause the mice to lose weight? Amlexanox was not altering their food intake, but Dr. Saltiel's group found that mice given amlexanox and a high-fat diet had a consistent increase in energy expenditure compared to mice not given the drug. Mice given amlexanox also had higher body temperatures, just like the genetically modified mice lacking IKK ϵ . These results supported the idea that amlexanox was increasing the amount of calories the mice burned, which could explain the weight loss and protection against weight gain while on a high-fat diet.

Releasing Energy: Restoring Fat Burning

One possible way to burn more energy involves activation of brown fat, a special type of fat that has been increasingly studied for its ability to burn calories, in part to help keep the body warm. (The body can also generate fat with similar characteristics, called beige fat, from the more abundant type of body fat.) Could amlexanox be affecting the mice's brown/beige fat? Dr. Saltiel and his colleagues studied the fat of mice receiving amlexanox and

SCIENTIFIC PRESENTATION

found that their fat tissue had characteristics of brown/beige fat, including increased amounts of proteins involved in fat metabolism and smaller stored fat droplets compared to mice not receiving the drug. This confirmed that amlexanox was directly affecting the activation of brown or beige fat to burn more energy.

Dr. Saltiel was also interested in how IKK ϵ factored into another metabolic problem commonly observed in obesity: a fat-burning defect called "catecholamine resistance." Catecholamines (including adrenaline) are stress hormones that help mobilize the "fight or flight" response to danger. Catecholamine resistance is a physiological state in which cells become resistant to these hormones, resulting in reduced breakdown of fats to provide energy in stressful situations. Although researchers had noted the presence of catecholamine resistance in obese adipose tissue for decades, the underlying mechanism of this condition was unknown. Dr. Saltiel's group discovered that elevated levels of IKK ϵ reduced the ability of certain receptors (called β -adrenergic receptors) in the fat cells of obese mice to respond to catecholamines. This reduction in β -adrenergic receptor activity resulted in reduced fat breakdown. However, treating the cultured adipose cells with amlexanox blocked this effect and restored sensitivity to catecholamines. Amlexanox also restored catecholamine sensitivity in obese mice.

This research demonstrated that IKK ϵ plays a role in glucose handling and in fat burning, and it explained further how blocking the activity of IKK ϵ could lead to weight loss in mice. These results may also have important implications to human health. Other research groups had showed that the β -adrenergic pathway was not as active in the fat cells of people with type 2 diabetes as in fat cells of people without diabetes.

Back in Balance: A Model for Restoring Metabolic Health

In conclusion, Dr. Saltiel described the current model of obesity as an unbalanced state. In a lean body, there is a balance between the β -adrenergic pathway, which encourages fat burning to mobilize energy resources, and insulin, which encourages fat storage. On a high fat diet, fat cells store energy and expand, creating stress. The stressed cells send out a chemical "cry for help," recruiting pro-inflammatory M1 macrophages. These immune cells release more signaling proteins, which activate IKK ϵ . IKK ϵ then mediates responses associated with obesity: both insulin resistance and catecholamine resistance. This combination results in a state of metabolic inflexibility. Dr. Saltiel suggested that this metabolic inflexibility might also be part of the reason that those attempting to lose weight can find shedding pounds more difficult over time, as the body responds to weight loss with a decrease in the breakdown of fats.

Dr. Saltiel's presentation clearly demonstrated how understanding the response to a high fat diet in mice can provide insight into human obesity and point to potential new treatments. If this research in mice can be translated into humans, the drug amlexanox—or other compounds that target IKK ϵ —might be a promising avenue for restoring balance to this system by inhibiting IKK ϵ and thus reducing inflammation, restoring energy balance, and decreasing weight. Dr. Saltiel and his research team have begun to test the effects of amlexanox on body weight and other metabolic measures in a preliminary study of a small number of people. His research group has recently found evidence that amlexanox may work through the same pathway in both mice and people. Dr. Saltiel hopes to perform larger clinical studies to determine if amlexanox might be a useful therapy for people with obesity.

Sisters Participate in Life-changing Clinical Trials—Testing Artificial Pancreas Technology for Managing Type 1 Diabetes



Michelle (left) and Paula

Sisters Paula and Michelle were diagnosed with type 1 diabetes 6 months apart 41 years ago when Paula was 12 years old and Michelle was 6. Taking cues from their parents, the sisters made the best of their situation. When they were children and had to take insulin shots, the sisters would save empty syringes (without needles), and “pass them out to our cousins as water guns,” they recall with a laugh.

People’s ability to manage type 1 diabetes has greatly improved since the sisters were children. However, type 1 diabetes is still an extremely burdensome and difficult disease to manage.

To keep blood sugar (glucose) levels within a healthy range, people with the disease (or parents of young children) must measure blood sugar levels with finger sticks or a continuous glucose monitor (CGM), calculate how much insulin to administer, and deliver that insulin via injection or pump. Paula and Michelle said that they typically measure their blood sugar levels four times a day, but it can be more often if their blood sugar is running too high or too low—then they need to monitor more often to make sure it comes back to a healthy range.

Additionally, while insulin therapy helps keep blood sugar from climbing too high, it brings with it the risk of potentially life-threatening episodes of low blood sugar (hypoglycemia). The risk of hypoglycemia greatly limits people’s ability to achieve recommended levels of blood sugar control—levels that have been shown by NIDDK-supported research to reduce the risk of long-term disease complications. Everyday experiences like eating, exercising, and illness can also affect blood sugar levels in unexpected ways, complicating people’s ability to predict changes in their blood sugar levels and determine how much insulin to take.

For these reasons, research is under way to develop new and improved tools to help people with type 1 diabetes manage their disease. Paula and Michelle are on the forefront of testing new technology—called an artificial pancreas—that could potentially help reduce the burden of managing the disease, as well as help people improve their blood sugar control.

PATIENT PROFILE

Signing Up for Their First Artificial Pancreas Clinical Trial

An artificial pancreas, or “closed-loop system,” currently being tested in research studies, is technology that would replace the function of the pancreas that does not work in type 1 diabetes: delivering insulin in response to blood sugar levels. It links three technologies: (1) a sensor, such as a CGM, that measures blood sugar levels and sends information to a computer; (2) an insulin delivery device, such as an insulin pump; and (3) a computer that calculates the amount of insulin needed and instructs the pump to deliver insulin.

Artificial pancreas technology would require minimal human input and mimic the exquisite control of blood sugar maintained by

a healthy pancreas. Thus, the technology could help people with type 1 diabetes achieve recommended levels of blood sugar control while preventing hypoglycemia, as well as alleviate the enormous burden associated with current management strategies—improving the health and quality of life of people with the disease.

Paula and Michelle have participated in two artificial pancreas trials at the University of Virginia (UVA), located in Charlottesville. It was Michelle who initially heard about their first trial because she liked to keep tabs on artificial pancreas research. Through www.clinicaltrials.gov, which is a service of the National Institutes of Health (NIH) with information on trials funded by NIH and other sponsors, “I found they had quite a few trials in Charlottesville for the artificial pancreas,” she says. She talked to one of the UVA researchers and found she was eligible to participate in an upcoming trial. She told Paula about it, with the hope that she could also participate, and both sisters enrolled in the trial. The trial was funded by JDRF—an organization that

has been a key research partner with the NIDDK to advance artificial pancreas research.

The technology they tested as part of that trial is called DiAs, short for “diabetes assistant,” and was developed by researchers at UVA. DiAs is an Android-based smartphone medical platform that serves as the “brains” of the operation; it is paired with a commercial CGM and insulin pump via Bluetooth. For safety reasons, during a clinical trial, the system allows the researchers to monitor real-time data from participants remotely so that they could intervene if the system doesn’t work properly. In

“closed-loop” mode, DiAs runs computer algorithms that, based on CGM data, predict when blood sugar levels will fall or

rise; in response, the system sends a signal to the insulin pump telling it to adjust insulin levels accordingly. The sisters had to input the number of carbohydrates (carbs) they were eating into DiAs, and to tell it when they were going to exercise, but otherwise the system took over control of managing their type 1 diabetes.

The first phase of the trial started in July 2014. “It was a 3-month trial that was to test the feasibility and safety of using an artificial pancreas at home,” explains Michelle. They first had to learn how to use the individual components of the artificial pancreas. Then, “we moved on to using the DiAs as an artificial pancreas at night. The last step was to use the system for 2½ weeks in 24-hour closed-loop mode,” she recalls. It didn’t take the sisters much time to adjust to using the new technology. “[It was] not hard to get used to it,” remembers Paula. “We like technology; we like new gadgets.” Michelle adds, “If you use a smartphone, you could definitely use an artificial pancreas.” In November 2014, they turned in their devices but then got them back in March 2015 when they started the

As Michelle explains, the artificial pancreas “was in complete control. You didn’t have to think about anything. You just put your carbs in there, and it gave you insulin, and you didn’t really have to worry.... It was great!”

PATIENT PROFILE

second phase of the trial, in which they used DiAs at home for 6 months in 24-hour closed-loop mode.

Artificial Pancreas Technology— A Life Changer

“We found that using the DiAs gradually changed our lives,” Michelle says. “It’s been a taste of freedom so to speak,” Paula adds. As Michelle explains, the artificial pancreas “was in complete control. You didn’t have to think about anything. You just put your carbs in there, and it gave you insulin, and you didn’t really have to worry.... It was great!”

One major benefit of using the system was that the sisters experienced many fewer episodes of

hypoglycemia. Michelle explains that if blood sugar levels drop extremely low during the day, “It pretty much wipes you out. Especially if it does it quickly, it completely wipes you out in terms of energy levels.” Paula adds: “Your brain function slows down, too.” The sisters further explain that blood sugar drops could happen quickly and unexpectedly. According to the sisters, the artificial pancreas helped protect against such episodes by keeping their blood sugar levels in a healthier range, so they didn’t experience the extremely low blood sugar levels they once did. If their blood sugar did drop, it dropped at a much slower rate, making it easier to manage.

Relief from the frightening and debilitating symptoms of hypoglycemia made driving less of a worry.

“Driving has always been a concern—I never wanted to have a low [blood sugar level] while driving, as the guilt would have eaten me alive if there had been an accident,” says Michelle.

However, because her blood sugar was being well-controlled by the artificial pancreas, “During the study, driving became just an everyday task,” she reports happily.

Use of the DiAs also helped the sisters sleep better, since low—as well as high—blood sugar levels could often interrupt their sleep. As Paula explains, “Before I got the DiAs, my sugar was dropping and then spiking in the night, and I would have to get up at least once during the night.” After starting on the device, though, she says that, “I was able to sleep through the night and my blood sugar levels were steady through the night and morning.” Michelle adds that, “I could get a full night’s sleep and would wake up ... refreshed and ready to take on the day.”

The ability of the artificial pancreas to control blood

sugar levels has positively affected other aspects of their lives. One of the things that the sisters liked most

“The [UVA] researchers are fantastic. They are striving to make our lives better.... I cannot sing their praises enough,” emphasizes Michelle. “They are a great bunch of people,” states Paula.

was having the ability to exercise and eat healthier, which, for them, resulted in losing some excess weight by the end of the trial. As Michelle explains, before using the device, “Every time I’d exercise I’d have to eat something,” because her blood sugar would drop so low. Even outside of exercise, the sisters had to eat extra calories during the day when their blood sugar dropped too low. However, the artificial pancreas protected against the very low blood sugar drops during exercise, so they didn’t have to eat a lot of food after exercising. And their blood sugar didn’t drop as often or as low when not exercising, so they didn’t need to consume as many extra calories during the day to raise it. “The fact that I wasn’t having to eat all the time ... gave me the ability to exercise and eat healthier,” Paula reports. Michelle states: “It feels good to only eat when you are hungry and not because you have to keep your blood sugar up.”

At the end of the trial, the sisters achieved impressive personal results. Paula’s hemoglobin A1c (HbA1c) level—a measure of average blood sugar control—improved from 7.7 percent to 6.6 percent.

PATIENT PROFILE

(The American Diabetes Association recommends that adults with type 1 diabetes aim for HbA1c levels less than 7.0 percent unless there is a reason to set a higher target.) Paula exclaims, "That is my all-time lowest HbA1c ever!" Michelle started with an HbA1c level of 6.9 percent, which was already at recommended levels, so she didn't think she'd see much change. However, her HbA1c level improved to 6.1 percent. Thus, both sisters achieved greatly improved blood sugar control with the artificial pancreas, while experiencing fewer episodes of hypoglycemia and improved quality of life.

The sisters recognized that the technology being tested in the trial was still a prototype device, and there are areas that need

to be improved. For example, the device would often lose Bluetooth connectivity, requiring them to reconnect the components manually. (There were safety mechanisms in place that alerted them to connectivity problems.) For safety reasons, there were also loud and frequent alarms, although they reported that the alarms lessened over time. "While this version was not the nirvana I had hoped for, it did allow me to relax a great deal," states Michelle. They also stress that the research team has been extremely willing to hear their feedback about how to make the system better and more user-friendly. "They are definitely wide open to any improvements that we suggested," the sisters report. Improving the "usability" of artificial pancreas technology is another important aspect of this research area.

Paula jokes that at the end of the trial, she and her sister weren't thrilled about turning in their artificial pancreas devices: "We had plans to run away with them," she laughs. More seriously, she adds: "While it was a sad day when I had to turn off the system and hand it over to the research team, I know that it brings us a step

closer to having it available to the public." She knows that today's clinical trials are paving the way to what scientists hope will be U.S. Food and Drug Administration (FDA) approval of the technology, which could lead to it being available to people with type 1 diabetes outside of a research setting. Of the trial, Michelle reports: "I could not have had a better experience."

Another Artificial Pancreas Trial— Project Nightlight

The sisters had such a great experience in their first trial that they eagerly signed up for a new

NIDDK-supported trial, called Project Nightlight, which began in spring 2016. In that trial, they are using "inControl," which

"While it was a sad day when I had to turn off the [artificial pancreas] system and hand it over to the research team, I know that it brings us a step closer to having it available to the public," says Paula.

is the commercial version of DiAs licensed by a start-up company called TypeZero Technologies; it uses the same algorithms as DiAs. Although the sisters only recently started the trial at the time they were interviewed for this profile, they already see two major improvements in the technology. First, the inControl system is much more user-friendly. Second, they aren't having the connectivity problems that they experienced in the first trial. Thus, the technology has advanced in the short amount of time between the two trials.

For the 11-month trial, Project Nightlight is examining different functionalities of the inControl platform. For example, for the first part of the trial, Paula and Michelle can only use the closed-loop mode starting at dinnertime and continuing overnight. The hardest part for them is that they want to use it all the time! However, they know that some people with type 1 diabetes may prefer to use closed-loop control during the nighttime only, so it is important to test the device at night only, as well as in 24-hour use.

PATIENT PROFILE

Helping Others Through Sharing Research Experiences

Paula and Michelle have active and busy lives. Paula is married to David and has a 28-year-old son, Nathan; she is a licensed practical nurse (LPN) and works for a public school system. Michelle is married to husband Gray and has two daughters, 18-year-old Abby and 16-year-old Maddie; she works from home as a web content manager for a government contractor. Even with their busy schedules, Paula and Michelle have made helping others one of their priorities. In addition to their family, work, and participating in artificial pancreas clinical trials, they have made time to share their experiences in their trials through a blog: www.diabeticsisters.net. One of the reasons they enrolled in the trials and started the blog was because they wanted to help others—including family members—who may not be as comfortable with new technology as they are; five other family members on both sides of their family have type 1 diabetes. “I really wanted to not only help them, but anybody else who might be scared about this [new artificial pancreas technology],” says Michelle.

As Michelle says and Paula echoes: “I personally cannot wait until this [artificial pancreas] equipment is released into the market ... so that I can use it 24/7 until a cure is found.... It’s truly life-changing.”

Hope Through Research

Paula and Michelle couldn’t say enough good things about the research team at UVA. “The researchers are fantastic. They are striving to make our lives better.... I cannot sing their praises enough,” emphasizes Michelle. “They are a great bunch of people,” states Paula. In addition to the group at UVA, there are several other research groups developing different artificial pancreas technologies. To propel research progress in this area, the NIDDK recently funded new advanced clinical trials testing different artificial pancreas systems. The goal is that these trials will pave the way toward generating data to satisfy safety and efficacy requirements for FDA approval of these systems. One of the recently funded trials is led by the UVA scientists.

With continued research, artificial pancreas technology represents a near-term approach that could transform the ability of people with type 1 diabetes to manage their disease with less burden while maintaining healthy blood sugar levels. As Michelle says and Paula echoes: “I personally cannot wait until this [artificial pancreas] equipment is released into the market... so that I can use it 24/7 until a cure is found.... It’s truly life-changing.”

PATIENT PROFILE

A Life in Public Health and Service to the Community Continues with Participation in the GRADE Study for Type 2 Diabetes



Earl with his wife, JoAnn

Earl, 71, is a self-described “news junkie” who enjoys travel and believes he was meant to go into public health. As a result, he spent over 34 years in a multi-faceted career helping people in Alabama and neighboring states to improve or manage their health—whether it was visiting communities during disease outbreaks, counseling cancer patients and their families, or encouraging people to join clinical trials. Thus, when he was diagnosed with type 2 diabetes, Earl was already keenly aware of the importance of clinical trials to improving health, with the upshot that he himself volunteered to be a participant in an NIDDK-supported clinical trial that is testing treatments for the disease—and, as he puts it, “Not a day have I regretted that. Not one day.”

Helping Others with Their Health

Earl grew up in a small, rural agricultural community near Birmingham, Alabama. He comes from a large

family of 11 siblings—five boys and six girls—all but one of whom are still living. While some have moved to other parts of the country, five of them, including Earl, still live in Birmingham, and they and their families get together regularly. “Those are the benefits that come from having a large family,” Earl notes happily. Earl himself has a son from his previous marriage and two stepsons from his current marriage.

After graduating from college, Earl joined the Air Force for 4 years, serving through basic training alongside one of his brothers, after which he was posted to Japan. It was during his time there, serving as an Air Force medic, that he feels his future career began. “I took care of patients coming back from Vietnam. Everything from bedside care, assisting the nurses and physicians, to direct patient care, even emergency [work],” Earl says, adding, “so I guess I was kind of destined, after that, to pursue some career here [in the United States] in ... public health after I left.”

Once he'd completed his active service in 1971, Earl immediately joined the Alabama State Health Department in communicable disease control. For 3½ years, he saw a lot of Alabama—“you know, ‘have car, will travel,’” he says jokingly. He could spend up to 2 to 3 weeks at a time in various, often rural, communities to locate affected individuals in disease outbreaks and get them treated by local physicians. Says Earl, “I think about those days, up and down the highway, sometimes 3,500 miles a month....” But, he adds firmly, “I wouldn't trade it for anything.”

In 1974, Earl joined the University of Alabama at Birmingham (UAB) Comprehensive Cancer Center as a counselor for patients and their families while in

PATIENT PROFILE

the hospital or in the outpatient clinic. He notes how being a part of the health care team was especially important for new patients and for people from small communities in Alabama—“patients often looked totally overwhelmed” after a visit from a large medical team, Earl remembers—and he became a “familiar face” for them, sometimes working with patients and their families for 2 to 3 years at a time. Earl admits that there were both high points and low points over the 18 years he was in this position, but emphasizes that, “probably, besides taking care of ... soldiers who were coming back from Vietnam, [this was] the most rewarding part of my career.”

During this time, Earl was also steadily working toward his Masters in Public Health degree in epidemiology. Between work and family, it was “one class per semester” for 11 years, says Earl. Then, while still working at UAB, he took on a position with a National Cancer Institute program called the Cancer Information Service (CIS). According to Earl, the CIS provided both a telephone-based service, which allowed people to call and get answers about their illness or about studies or programs nearby that might be able to help them, and an outreach program. As an outreach coordinator, Earl spent a lot of time encouraging people across Alabama and two other states to volunteer for clinical studies, often visiting their communities in person.

Health Challenges and Putting Knowledge into Action

At the same time as he was working with such dedication to help others with their health, Earl was hit by some health problems of his own. Once an avid tennis player, he had to exchange it for racquetball when he was diagnosed with psoriatic arthritis, a

painful and potentially debilitating condition, in his thirties—“jarring news,” Earl says, though he feels he’s been fortunate with his outcomes and has not had to take any medication for the arthritic pain for a couple of decades. More problematically, he developed high blood pressure (hypertension) in his mid-thirties, which worsened as he got older and was a challenge for him and his physician to get under control. Earl has a strong family history of high blood pressure, which he believes contributed to the early deaths of his father and the brother he served with in the Air Force.

[Earl] volunteered for an NIDDK-supported clinical trial that is testing treatments for [type 2 diabetes]—and, as he puts it, “Not a day have I regretted that. Not one day.”

In the years following those diagnoses, Earl’s activity levels decreased, in large part due to arthritic pain, such that he even had to give

up racquetball. Over time, his weight crept up, with his highest weight hitting 262 pounds. About 12 to 14 years ago, when he was in his fifties, Earl’s doctor noticed that his numbers from a blood test that indicates risk of type 2 diabetes were creeping up, too. In 2007, Earl was diagnosed with the disease. Seven years later, with his diabetes still not under optimal control despite medication and some weight loss, Earl decided to do what he’d encouraged so many others to do: search to see if there was a clinical study at UAB that could help with his type 2 diabetes—and that is how he found the GRADE Study.

Type 2 Diabetes and the GRADE Study

In type 2 diabetes, the body becomes resistant to the action of insulin—the master hormone in the body controlling blood sugar (glucose) levels—and the pancreatic cells that produce insulin don’t function normally, causing blood sugar levels to rise. This, in turn, can cause damage to blood vessels, organs, and nerves throughout the body. Risk factors for type 2 diabetes include older age, obesity, and a family history of the disease; certain racial and

PATIENT PROFILE

ethnic groups in the United States, including African Americans, are also at greater risk.

Research studies have shown that keeping blood sugar levels as close to a healthy range as possible can help stave off or delay progression of diabetes health complications, such as kidney disease and blindness. Currently, health care providers have several different options to help people with type 2 diabetes control their blood sugar levels. The drug metformin is the first-line medication used to treat type 2 diabetes, but if or when metformin is insufficient to manage the disease, health care providers can add a second drug that has been

approved for use with metformin by the U.S. Food and Drug Administration. But which one?

While short-term studies have demonstrated

efficacy of different drugs in combination with metformin, long-term studies have not been performed to see which combinations work best over time, with the least side effects, and in whom.

To address these critical questions, the NIDDK launched the clinical trial, "Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness (GRADE) Study," also referred to as GRADE. The primary aim of the GRADE Study is to compare how four different drugs, when given in combination with metformin, affect blood sugar levels, diabetes complications, and quality of life—as well as to identify any adverse outcomes they might have. The study will evaluate each participant's health for approximately 5 years. Recruitment for the study started in June 2013, with the goal of enrolling 5,000 volunteers around the country who have been living with a diagnosis of type 2 diabetes for less than 10 years and, if on medication, have only been using metformin.

At the initial screening, a potential volunteer's degree of blood sugar level control is measured by a test called the HbA1c test, to see if it meets the threshold for the study. That threshold is an HbA1c measurement of 6.8 percent or greater. (The normal range for someone without diabetes is an HbA1c of 5.7 percent or less.) A person who meets this screening criterion then receives additional medical tests over several weeks, and is given information about the study and about diabetes. After this period, eligible volunteers whose HbA1c levels are between 6.8 and 8.5 percent are randomly assigned to one of the four drug

treatment combinations (a treatment "arm" of the study) being tested in GRADE.

The four medication classes and the specific drugs being tested in combination with metformin are:

Says Earl, "I do feel better, there's no question about it. In fact, there was a time I would get out to wash the car and I would be tired after washing the car. Now, I can wash two cars and still have some energy left to do something else. And I don't get tired climbing the stairs. It's been remarkable."

- Sulfonylurea (glimepiride, brand name Amaryl®), which increases insulin levels directly;
- DPP-4 inhibitor (sitagliptin, brand name Januvia®), which indirectly increases insulin levels by increasing the effect of a naturally occurring intestinal hormone;
- GLP-1 receptor agonist (liraglutide, brand name Victoza®), which increases the amount of insulin in response to nutrients; and
- a long-acting insulin (glargine, brand name Lantus®).

Two of the medications, the sulfonylurea and the DPP-4 inhibitor, are taken orally, while the other two are taken by injection under the skin. Metformin (Glucophage®) is also taken orally. All medications are taken every day. GRADE volunteers receive the study medications for free, and attend at least four medical visits per year with their GRADE clinical study site staff so that health outcomes, including blood sugar levels, blood pressure, effects on risk factors for cardiovascular disease, any

PATIENT PROFILE

adverse events (such as blood sugar levels dropping too low), and quality of life, can be measured or assessed. The study will also be examining issues of cost and cost-effectiveness of the treatments.

Because blood sugar control is the focus of GRADE, the primary outcome of interest is how long a treatment helps an individual to achieve and/or maintain control of blood sugar levels at a level below an HbA1c of 7.0 percent. (The American Diabetes Association recommends that adults with type 2 diabetes aim for HbA1c levels less than 7.0 percent unless there is a reason to set a lower or higher target, hence this target in the GRADE Study.) The GRADE Study scientists anticipate that results from comparing the different treatments should begin emerging sometime after 2020.

Making the GRADE

When Earl volunteered for the GRADE Study in September 2014, his HbA1c numbers met the threshold criteria. Within a few weeks, he was fully enrolled in one of the four treatment arms of the GRADE Study. He admits that he wasn't so excited when he found that he was randomly assigned to one of the injectable medications, the GLP-1 receptor agonist, liraglutide. "I was truly hoping for the option that would allow me to take the pills by mouth," Earl says. However, he credits the clinic for demonstrating the way to do the injection, noting that after that, "I came home and I never had any regret, from day 1."

Because he can tolerate it, Earl is taking the maximum dose of liraglutide being used in the GRADE Study and describes his daily medication regimen as follows: "I take the [first dose of] metformin and my hypertension medication in the morning, when I get up and have

a cup of coffee and piece of toast or whatever it is.... Then somewhere a little before I leave—often during the day, that's around noon or 1 o'clock ... I'll go ahead and take the [liraglutide]. And if ... for some reason I rush out the door and forget about it, I take it when I get home, before I set up and start watching my news programs.... And then I take the second dose of my metformin."

Feeling the Health Benefits at Home and Beyond

When Earl first entered the GRADE Study in September 2014, his HbA1c was 7.3 percent, and he weighed 254 pounds. Two years later, his HbA1c had dropped to 5.5 percent and he was 196 pounds. "I can't remember the last time I was under 200 pounds," says Earl.

Earl says he thinks his weight loss was gradual. "I didn't notice it as much at first, until I started noticing my belt did not fit in the same place," he says. His weight loss didn't go unnoticed by family and friends, however. At a big family reunion in Illinois over the

summer, "my sisters and brother in this area [Birmingham] who see me on a fairly regular basis were raving about how much weight I had lost, so I guess

When Earl talks about the GRADE Study, he has no end of praise. "I cannot say enough about the people in the clinic and how fantastic they've been every time—they have really and truly made me feel like a family member every time I've been there," says Earl.

that was positive reinforcement. Especially my two youngest sisters ... they just couldn't quit talking about how much weight [I'd lost], and how good I looked," says Earl, adding "I guess even if you don't think of yourself as being very vain, it certainly doesn't hurt to hear that."

He also has a lot more energy. Says Earl, "I do feel better, there's no question about it. In fact, there was a time I would get out to wash the car and I would be tired after washing the car. Now, I can wash two cars and still have some energy left to do

PATIENT PROFILE

something else. And I don't get tired climbing the stairs. It's been remarkable."

When asked about how he feels on the study medication in general, Earl says he's noticed a decrease in appetite and that he doesn't crave the same kinds of foods he once did—for example, fried chicken wings.

"Just one day, it just seemed my appetite for it was gone." He adds, "Even in the morning, I don't

care that much for bacon anymore.... I just don't have much of an appetite beyond one egg or grapefruit." Now, he says, he enjoys soups, and can "eat [beans] for days."

The positive health changes Earl has experienced so far during his participation in the GRADE Study have also had a positive impact on other parts of his life. Although he retired from UAB in 2005, Earl has continued to work in another area that he's been involved in for over 20 years, first with one of his brothers and now with his son—real estate. He says the business is "in an area [of Birmingham] that is low income, and so we feel like every time we buy a house and fix it up, we're making a contribution to the community." Moreover, Earl adds, "We've established a relationship with an organization here ... over the last 6 months [that helps] women who are homeless to find a place to live ... just another way of giving back." As he goes out daily during the week, "driving out to look at one of [the] properties, or just driving around to meet a tenant who wants to look at [one]," his weight loss and improved energy contribute to a better day.

Spreading the Word About the GRADE Study

When Earl talks about the GRADE Study, he has no end of praise. "I cannot say enough about the people in the clinic and how fantastic they've been every

time—they have really and truly made me feel like a family member every time I've been there," says Earl. He recalls one example, a luncheon hosted by the clinic for GRADE volunteers in 2015, and how the UAB GRADE Study coordinator, Dana Golson, R.N., CDE, was there and welcomed every single person who attended as they came in the room. "If there's

an ambassador for GRADE, she's it," says Earl.

Because of his own past work in

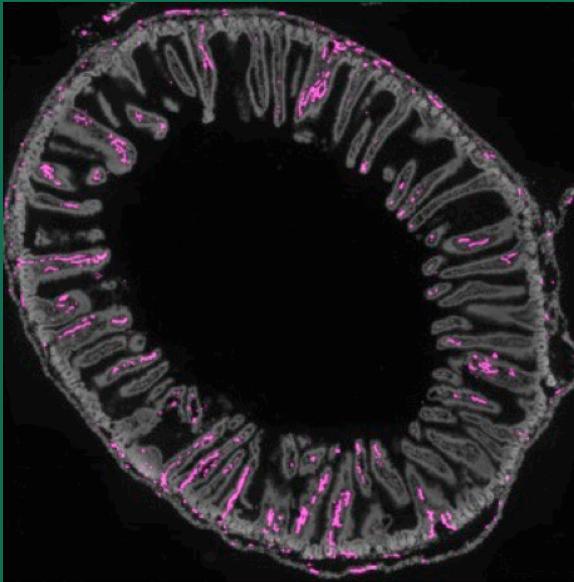
public health and the challenges he encountered in encouraging people to join clinical trials, Earl is also happy to see the diversity of GRADE participants in his own clinic group, including many African Americans, observing that, in clinical trials, "you need people who [represent] diverse populations in the study to come up with a conclusion that is valid."

Earl also continues to be a health ambassador himself. While no one else in his family has diabetes, he knows of several friends who do, and says he would encourage people to join the GRADE Study "in a heartbeat." He talks about the GRADE Study at church, with friends and family, and, Earl adds, "I have a group of my long-time [college] fraternity brothers, we get together on a monthly basis ... and I tell them about the study."

When asked what is the strongest personal impression he'd use to encourage people to volunteer for the GRADE Study, Earl puts it simply and succinctly: "Feeling better, I think is what I'd focus on—on feeling better."

While no one else in his family has diabetes, [Earl] knows of several friends who do, and says he would encourage people to join the GRADE Study "in a heartbeat."

As of January 2017, the GRADE Study is still actively recruiting participants at clinical sites across the country. For more information about the study and how to participate, please see <http://gradestudy.com/>



How does the brain know when the stomach and intestines contain food to be digested? Researchers recently gained insights by studying the vagus nerve (the tenth cranial nerve), which transmits information from the gut to the brain through many different nerve cells (neurons). They discovered that some of these cells, called GLP1R neurons, sense when the stomach and intestines have stretched in size to accommodate a meal just eaten, while others, called GPR65 neurons, detect nutrients to be digested. As shown in this image of a section of mouse intestine, fibers from GPR65 neurons, labeled in pink, spread throughout intestinal structures that absorb nutrients.

These fibers are thus ideally situated to detect food, so that the neurons can signal the brain accordingly. Fibers from GLP1R neurons permeate other parts of the gut. Described further in this chapter, this study sheds new light on brain-gut communication.

Image courtesy of Dr. Stephen Liberles and Erika Williams, Harvard Medical School. Image credit: Dr. David Stochlic. Reprinted from Cell, vol. 166, Williams EK, Chang RB, Stochlic DE, Umans BD, Lowell BB, Liberles SD, Sensory neurons that detect stretch and nutrients in the digestive system, pages 209-221, copyright 2016, with permission from Elsevier.

Obesity

Obesity has risen to epidemic levels in the United States. Individuals with obesity may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission. More than one third of U.S. adults are considered to have obesity based on body mass index (BMI), a measure of weight relative to height.¹ Approximately 17 percent of children and teens ages 2 through 19 also have obesity, and thus may be at increased risk for developing serious diseases both during their youth and later in adulthood.^{2,3} Obesity disproportionately affects people from certain racial and ethnic groups and those who are socioeconomically disadvantaged.

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

The NIDDK supports a multi-dimensional research portfolio on obesity, spanning basic, clinical, and translational research. NIDDK-funded studies investigate a variety of approaches for preventing and treating obesity. These span behavioral and environmental interventions in families and in health care and other settings, using a variety of approaches and technologies; surgical interventions; and combinations of strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight have continued to spark new ideas for intervention approaches. To help bring research results to those affected by obesity and their families, health professionals, and the general public, the Institute sponsors health information programs.⁴

The NIDDK also continues to play a leading role in the NIH Obesity Research Task Force. The NIDDK Director co-chairs the Task Force along with the Directors of the National Heart, Lung, and Blood Institute and the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The Task Force

includes representatives from these and numerous other NIH Institutes, Centers, and Offices.

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

THE BRAIN'S REGULATION OF APPETITE AND DIGESTION

New Insights into How the Brain Handles Hunger:

Researchers have discovered new details about how the brain controls food intake in mice, suggesting a possible therapeutic target for curbing appetite. In the central nervous system, hormones called melanocortins can activate signaling through the melanocortin 4 receptor (MC4R), affecting metabolism, food intake, and calorie burning (energy expenditure), as well as other physiological factors such as blood pressure. Genetic changes that inactivate the gene for MC4R can cause severe obesity. Without MC4R, food intake and body fat increase, while energy expenditure decreases and the body becomes less able to respond to the hormone insulin. Unfortunately, past attempts to activate MC4R's effects as a potential obesity treatment also resulted in higher blood pressure, limiting its use as a therapy.

¹ Flegal et al. *JAMA* 315: 2284-2291, 2016.

² Ogden et al. *JAMA* 315: 2292-2299, 2016.

³ 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).

⁴ NIDDK Weight-control Information Network, <http://win.niddk.nih.gov/index.htm>

A team of scientists thus sought new insights into MC4R and proteins it partners with, as possible avenues to new treatment strategies. MC4R was known to work through a signaling protein called $G_{5\alpha}$ to affect energy expenditure and glucose metabolism in the brain. However, $G_{5\alpha}$ did not appear to be responsible for MC4R's effects on reducing food intake, which originate in a different part of the brain, called the paraventricular nucleus (PVN). Thus, scientists hypothesized that another MC4R-triggered pathway was involved in food intake. To determine what this pathway might be, researchers investigated MC4R's interactions with a pair of other signaling proteins, which they referred to collectively as $G_{q/11\alpha}$. To determine if $G_{q/11\alpha}$ plays a role in regulating food intake, the researchers created genetically engineered mice lacking $G_{q/11\alpha}$ in the PVN and looked at the mice's behavior, weight, and metabolism. Mice lacking $G_{q/11\alpha}$ ate more food and, later, developed severe obesity compared to mice that still produced $G_{q/11\alpha}$. This increased weight was especially prominent in female mice. Additionally, a chemical that usually reduces food intake when injected into the PVN had a reduced effect in mice lacking $G_{q/11\alpha}$ in the PVN, demonstrating that $G_{q/11\alpha}$ was involved in curbing appetite. The mice lacking $G_{q/11\alpha}$ also developed elevated cholesterol levels. However, the lack of $G_{q/11\alpha}$ in the PVN had no effect on heart rate and blood pressure. These results confirmed that melanocortin's effects on food intake and cholesterol levels in the PVN were mediated through $G_{q/11\alpha}$, a different pathway than that which mediates melanocortin's effects on blood pressure.

Overall, these results provide new clarity to the question of how hunger and obesity are regulated by the brain. They also offer new avenues for possible therapeutic interventions, since therapies that are specific to the $G_{q/11\alpha}$ pathway may be able to suppress appetite without unwanted cardiovascular side effects.

Li Y-Q, Shrestha Y, Pandey M, ... Weinstein LS. $G_{q/11\alpha}$ and $G_{5\alpha}$ mediate distinct physiological responses to central melanocortins.

J Clin Invest 126: 40-49, 2016.

The Inner Workings of a Brain Cell That Drives

Eating and Weight Gain: Seeking insights that could lead to novel obesity treatments, scientists discovered a molecular pathway in mouse brain cells that begins with activation of cell-surface proteins, called G protein-coupled receptors (GPCRs), and ends with the cells' release of a

powerful appetite-inducing molecule, AgRP. In planning their research, the team of scientists chose to study certain cells in the brain, called AgRP neurons, that produce several factors to promote food intake, including their namesake AgRP molecules. The scientists focused on the cells' GPCRs because these types of proteins transmit important signals in cells throughout the body to maintain health. GPCRs are also the targets of many medications on the market today for many conditions—and are thus tantalizing prospects for potential future drug development.

The researchers decided to investigate one type of GPCR, the G_s -coupled version, to elucidate its function in AgRP neurons. However, because there are many different forms of GPCRs in many different cells, including multiple forms even within AgRP neurons, they needed a way to zoom in on these particular GPCRs. They adapted a technique, developed previously by others, to create a “designer” G_s -linked GPCR in mice that could only be produced in AgRP neurons and could only be activated by a particular chemical. Then, they tested the effects of this GPCR on food intake, using male mice that had the designer G_s -coupled GPCR in their neurons. Would administering the activating chemical to mice that had just eaten—and thus should be full—cause them to start eating again? It did. Following a single dose of the chemical to activate the G_s -coupled GPCR, the mice not only greatly increased their food intake over the next several hours, but they also continued overeating for the three days of the experiment. Their voracious appetite was not without consequences—the mice gained weight. With further experiments, the researchers mapped additional molecular steps along the pathway from the initial G_s -GPCR activation to the ultimate release of appetite-inducing AgRP molecules. Finally, they confirmed that the insights gained from their designer G_s -GPCR also applied to a native G_s -GPCR in AgRP neurons.

This study brings to light the role of G_s -coupled GPCRs in the brain's regulation of food intake and body weight. In this and previous research on AgRP neurons, the scientists also investigated another type of GPCR, and found that it, too, prompted mice to eat, but through a different molecular pathway. With multiple ways to provoke eating, the inner workings of these cells may pose a challenge to well-intentioned dieters, but they also present an opportunity. If future research reveals similar findings in people, scientists could develop drugs that interfere with steps along these brain cell pathways,

with the goal of suppressing appetite to help individuals lose excess weight.

Nakajima K, Cui Z, Li C, ...Wess J. G_s-coupled GPCR signaling in AgRP neurons triggers sustained increase in food intake.

Nat Communications 7: 10268, 2016.

A Nutrient Sensor in Brain Cells Regulates Feeding:

New research in mice has revealed a key enzyme that acts as a control switch for feeding. Obesity is a worldwide epidemic and a major public health concern. Maintaining energy balance—or the balance between calorie intake and calorie burning—is crucial to keeping a healthy weight and preventing obesity. In addition to lifestyle habits, such as diet and exercise, numerous genes and signaling molecules influence energy balance. The brain regulates food intake by responding to signals in the body, but how the brain interprets these signals is unclear. Previous studies have shown the enzyme O-GlcNAc transferase (OGT) is important in the development of brain cells (neurons) and is regulated by nutrients and insulin, but its precise role in the adult brain was unknown. In this study, researchers investigated the function of OGT in male mice by knocking out the gene that encodes OGT (“OGT knockouts”) within a very specific set of neurons in the brains of adult mice. They then compared food intake and weight in mice without OGT to normal mice.

They found that brain-specific loss of OGT caused a rapid weight gain in mice. Within 3 weeks, the genetically modified mice tripled their body fat. Although these mice ate only as frequently as their unmodified counterparts, they ate more at each meal. The researchers observed that if they restricted food access, the knockout mice maintained a normal weight. However, when free access to food was reintroduced, the mice quickly became obese. When they examined the brains more closely, they found that the loss of OGT was occurring in a particular region of the brain known to be involved in appetite regulation, called the paraventricular nucleus (PVN) of the hypothalamus. Further studies showed that upon deleting OGT, the PVN neurons became far less active, as if they had been silenced. Because disrupting OGT in PVN neurons inhibited the activity of these cells and caused the mice to overeat, the team reasoned that stimulating these cells would have the opposite effect and decrease food intake. To test this theory, they genetically manipulated PVN cells further

in knockout mice to produce light-sensitive proteins on their membranes. When they stimulated the cells with a beam of light, the cells became activated and fired signals to other parts of the brain, causing a reduction in food intake.

Taken together, these findings identify OGT in PVN neurons as a potential off-switch to control overeating. While more studies are needed to determine if these cells work the same way in humans, OGT in PVN neurons represents a new potential therapeutic target for human obesity.

*Lagerlöf O, Slocomb JE, Hong I, ...Huganir RL. The nutrient sensor OGT in PVN neurons regulates feeding. *Science* 351: 1293-1296, 2016.*

How the Brain Knows When the Stomach Has Stretched and There Is Food To Digest:

Exploring a biological data cable that transmits information from the gut to the brain, researchers discovered nerve cells in mice that detect nutrients to be digested, and other nerve cells that sense when the stomach and intestines have stretched in size to accommodate food just eaten. To regulate digestion and other processes, many nerve cells (neurons), bundled together to form the vagus nerve, monitor organs throughout the body and report back to the brain. Long fibers from this group of neurons permeate the stomach, intestines, or other organs at one end of the vagus nerve, while fibers at the other end reach up to connect to the brain. It has not been clear, however, which of these neurons transmit which signals.

To identify cells within the vagus nerve that carry different signals, a team of researchers developed a strategy, using a set of fluorescent biological tags, for observing individual neurons and their activity. They first genetically engineered neurons in male and female mice to produce a fluorescent protein that would glow when the cells were activated—that is, transmitting signals—and then examined hundreds of neurons under different conditions. The researchers found that some neurons were activated in response to stretch in the stomach and intestines, while others were activated by the presence of nutrients, as seen by their fluorescent glow. Next, the researchers sought to reveal the identity of these neurons. They focused on a few types of neurons they suspected might play a role based on signaling proteins they produced, called GLP1R and GPR65. The researchers genetically

engineered neurons so they would glow when activated, and used additional fluorescent tags, which glowed in a different color, to mark cells containing GLP1R or GPR65, so they could spot these among other neurons. With other techniques, they also mapped the nerve fibers of these cells in the digestive tract. They found that GLP1R neurons projected their fibers into stomach and intestinal muscle, and that these cells became activated in response to stomach and intestinal stretching. By contrast, GPR65 neurons spread their fibers through intestinal structures that absorb nutrients and respond to food in the intestine. Thus, GLP1R and GPR65 neurons monitor and transmit different signals related to digestion. In other experiments, the researchers found that both types of neurons, when activated, could affect gut motility—the contractions and pressure that help digestion.

By identifying which neurons sense nutrients and stretch in the digestive tract and affect gut motility, this research sheds new light on the connections between body and mind. This study may also lead to new insights into digestive disorders.

Williams EK, Chang RB, Storchlic DE, Umans BD, Lowell BB, and Liberles SD. Sensory neurons that detect stretch and nutrients in the digestive system. Cell 166: 209-221, 2016.

NEW DIRECTIONS IN OBESITY TREATMENTS

Brain Stimulation with Electric Current Leads to Changes in Food Consumption and Body Weight in a Preliminary Study of Adults with Obesity: In an intriguing preliminary study, researchers found that a method for stimulating brain activity with electricity, transcranial direct current stimulation (tDCS), affected food choices of people with obesity and led to a small amount of weight loss over several days. Given the challenges of weight loss with lifestyle changes alone, researchers have begun to explore novel treatment approaches, beyond medication and bariatric surgery, that could be combined with or facilitate healthy eating and physical activity. These approaches include modulation of brain activity, because the brain plays major roles in hunger and satiety, in the feeling of reward when eating delicious food, and in other aspects of eating.

In this study, a team of researchers tested tDCS, a non-invasive but still experimental method for

stimulating activity in specific regions of the brain with electricity, to see if it might help people with obesity. Nine volunteers, including both men and women, resided in one of NIDDK's intramural metabolic research centers on two separate occasions for the study, where they received either tDCS or a sham (control) treatment, and where their food consumption could be carefully examined. For the tDCS, the researchers placed electrodes on the participants' heads to target a part of the brain involved in behavioral regulation and reward. During their visits to the research center, which lasted 8 days each, the volunteers spent the first 5 days on a weight-maintaining diet that was prepared for them. For the next 3 mornings, they were randomly assigned to receive either tDCS or the sham treatment. After their treatments, they ate all of their food from special computerized vending machines; they could choose whatever they wanted to eat and drink from the vending machines, and could eat whenever and as much as they wished. Their food choices, the amounts they consumed, and their body weights were recorded. Five of the volunteers received an inactive form of tDCS on their first visit to the research center, and active tDCS on the second visit. These individuals consumed significantly fewer calories from fat and soda and lost more weight during the visit in which they received the active tDCS. The four volunteers who received the sham treatment on both visits to the center did not experience these changes.

This study provides preliminary evidence that tDCS might affect food consumption and help reduce body weight in people who are obese. The results are consistent with several previous small studies that found potential effects of tDCS on eating behavior. However, because the current study included only a small number of participants, and the effects of the treatment were monitored for only 3 days, much more research would need to be done to test the safety and effectiveness of this procedure for weight loss.

Gluck ME, Alonso-Alonso M, Piaggi P,...Krakoff J. Neuromodulation targeted to the prefrontal cortex induces changes in energy intake and weight loss in obesity. Obesity 23: 2149-2156, 2015.

Naturally Occurring Compound Shows Potential as a Treatment for Obesity and Diabetes: Researchers have used an innovative drug-discovery approach to identify a naturally occurring compound, called withaferin A, that mitigates obesity and its metabolic

effects in mice. Fat cells secrete a hormone called leptin, which signals to the body to stop eating when energy stores are sufficient. When leptin was first discovered in the 1990's, it was thought that the hormone may be useful to treat obesity. However, except in rare cases of obesity caused by leptin deficiency, obese individuals actually have high levels of leptin, but they are resistant to leptin's actions. Thus, in obese individuals who are leptin resistant, the hormone is unable to curb appetite, resulting in overeating and additional weight gain. In new research, scientists were interested in identifying potential therapies that could combat leptin resistance and thus promote weight loss. Rather than using a common approach of screening thousands of potential drugs against a single molecular target to identify promising compounds for further testing, the researchers used an innovative drug-screening approach by building on their previous research that identified a naturally occurring compound, called celastrol, which increased leptin sensitivity and promoted weight loss in mice. They looked for compounds that induced a similar gene expression profile—*i.e.*, the genes that are turned on and off—in cells as when they are treated with celastrol. They reasoned that such compounds may similarly improve leptin sensitivity. This approach led to the discovery of withaferin A.

The researchers next examined whether treatment with withaferin A, like celastrol, could reduce body weight in different male mouse models. Withaferin-A treatment of mice that were obese and leptin-resistant because of eating a high-fat diet led to a 23 percent reduction in body weight and a 35 percent reduction in fat mass compared to control mice; treated animals also ate substantially less food. Withaferin-A treatment also resolved the animals' fatty liver disease, a condition associated with obesity. In contrast, withaferin A did not reduce the body weight of lean mice, which are not leptin resistant, and only marginally affected the body weight of mice that do not make leptin because of a genetic mutation, and thus would not be expected to be affected by a therapy that improves leptin sensitivity. These results suggest that, like celastrol, withaferin A improves leptin sensitivity in mice and promotes weight loss. Further experiments showed that withaferin A (unlike celastrol) also had anti-diabetic properties. For example, withaferin-A treatment improved glucose tolerance, insulin sensitivity, and blood glucose levels

in the mice that were obese and leptin-resistant as a result of eating a high-fat diet. Surprisingly, withaferin A also improved glucose tolerance and blood glucose levels in the mice lacking leptin, suggesting that withaferin A's effects on glucose metabolism are independent of its ability to improve leptin sensitivity. This research identifies a novel compound that improves leptin sensitivity, promotes weight loss, and has beneficial effects on glucose metabolism in mice. Additional research is needed to determine the cellular mechanisms by which withaferin A exerts these effects, as well as whether it will have similar benefits in people without causing unwanted side effects. Future research could build on these promising findings in mice, as well as on the screening approach used by the scientists as a way of identifying other potential therapies for obesity and diabetes.

Lee J, Liu J, Feng X, ... Ozcan U. Withaferin A is a leptin sensitizer with strong antidiabetic properties in mice. Nat Med 22: 1023-1032, 2016.

Custom-made Fat Tissue That Burns Calories:

In research that might lead to a new obesity and diabetes treatment approach, scientists developed a novel technique in mice for directing stem cells from body fat to grow in special gels and form fat tissue that burns—rather than stores—calories. Based on earlier findings that some types of body fat, called brown and beige fat, can generate heat by burning stored calories, researchers have proposed various strategies for creating more of these types of tissues to reduce excess weight and boost metabolism. Pursuing one such strategy, a multidisciplinary research team sought to grow beige fat tissue in the lab and test whether it would improve weight and health in mice.

They began by extracting stem cells from white fat tissue, the more abundant type of fat best known for storing calories. Next, they devised a way to coax these cells into becoming beige fat, taking into account the importance of a cell's surrounding environment in determining its fate. In the body, critical signals come not only from molecules that enter into cells, but also from the biological structures on which cells sit, including the proteins on these structures. Thus, to grow the stem cells, the researchers developed a special gel matrix that included fragments of proteins they had carefully selected to help guide stem cell maturation into beige fat. After immersing the stem cells in chemicals known to induce beige

fat characteristics, the researchers mixed the cells with the gel components to help seal their fate, and transplanted the mixture into male mice. The technology worked. Cells grown in the special gel turned on a key beige fat gene, *UCP1*, used for generating heat from calorie burning, and did so more effectively than cells grown in other ways. Mice transplanted with this new beige fat gained less weight than other mice on a high-fat diet. They also had less fat in their bloodstream; higher body temperatures after exposure to cold; and improved blood sugar levels, a sign of reduced risk for diabetes.

In the future, scientists could test this new technology to see whether it works with human cells. If it does, a person's own excess fat tissue might one day be used as a source of stem cells for generating beige fat—turning a problem into a potential solution.

Tharp KM, Jha AK, Kraiczy J,...Stahl A. Matrix-assisted transplantation of functional beige adipose tissue. Diabetes 64: 3713-3724, 2015.

INSIGHTS INTO WEIGHT LOSS MAINTENANCE

Research Reveals Persistent Metabolic Slowing as Potential Catch-22 in Maintaining Significant Weight Loss:

A long-term follow-up study of participants in a televised weight loss competition suggests that there is a persistent change in how the body handles calories that can interfere with efforts to maintain weight loss. Scientists in the NIDDK Intramural Research Program originally studied metabolic changes in 16 extremely obese men and women who lost weight through intensive diet and exercise in the televised “The Biggest Loser” competition. They have now conducted a follow up study with 14 of these people 6 years after the end of the 30-week competition. A key finding in the original study was that, between the start and end of the competition, participants’ weight loss was accompanied by greater than predicted and substantial reduction in their resting metabolic rate (RMR)—a measure of the minimum amount of calories the body will burn per day. Lowering RMR is a way for the body to resist weight loss and to be able to function on fewer calories, and is advantageous in circumstances such as starvation. However, in the context of losing excess fat weight, this metabolic adaptation may contribute to weight regain, especially if it persists.

In the new study, the NIDDK scientists obtained data on body composition so that—using an equation developed in the original study that also takes into account factors such as age and gender—they could calculate a new predicted RMR for each person. They also measured each person's actual RMR. When they compared the data from the beginning of the competition, the end of the competition, and 6 years later, they found that all but one participant had regained at least some of their lost weight. At the same time, participants’ RMRs had, on average, remained at the same reduced level seen at the end of the competition, rather than increasing as would be predicted with weight regain. This alarming result suggests that metabolic adaptation following diet- and exercise-induced weight loss persists and does not fully reverse even as weight is regained, adding to people's struggle to maintain weight loss. Encouragingly, however, when examining individual results, the researchers found that the persons who maintained greater weight loss at the 6-year mark also experienced greater ongoing metabolic slowing—suggesting that the observed RMR adaptation does not completely counter weight loss. Maintaining a lower body weight nonetheless requires continued attention to physical activity and dietary changes, given the body's tendency to burn fewer calories after weight loss.

Fothergill E, Guo J, Howard L,...Hall KD. Persistent metabolic adaptation 6 years after “The Biggest Loser” competition. Obesity 24: 1612-1619, 2016.

DISCOVERIES PROVIDE INSIGHTS ON EATING BEHAVIOR

How What You Eat Can Affect How Much You Eat:

Scientists identified one way the gut microbiome influences obesity and metabolism. A link between the bacteria that populate the intestines (part of the gut microbiome) and obesity had been previously discovered, but the details of how the microbiome influenced body weight were not known. To delve into this question, researchers built on a previous observation: changes in the amount of short-chain fatty acids (by-products of digestion in the gut) can be associated with overfeeding, obesity, and metabolic syndrome (factors that increase risk of heart disease and diabetes). In this new study, the scientists found that male rats fed a high-fat diet showed a striking increase in the amount of acetate, a short-chain fatty acid, in their bodies, and became

insulin resistant, a condition associated with metabolic syndrome. Determining the origin and consequences of the increase in acetate resulted in an exciting discovery of how the gut microbiome affects metabolism.

By measuring the acetate in tissues of the rat, the scientists found the highest amount in the gut; treating the rats with antibiotics to kill the gut bacteria, or removing the colon (part of the gut), reduced the amount of acetate dramatically. Consistent with previous research, they also found that rats fed a high-fat diet had a mix of bacteria in their microbiome that was somewhat different from the gut bacteria of rats fed a normal diet. A fecal transfer—transplanting the gut microbiome from rats eating the high-fat diet into rats on a normal diet—also transferred the increase in acetate production. Together, these observations indicate that the gut microbiome was responsible for generating the increased acetate. To determine the chronic effects of increased acetate, rats on a normal diet received acetate infusions for 10 days. After this period, the rats had increased insulin secretion by the pancreatic β (beta) cells in response to insulin, were insulin resistant, and more than doubled their daily caloric intake and weight gain. Interestingly, the researchers discovered that the acetate stimulated the parasympathetic nervous system through the brain.

These results suggest a model: exposure to a diet high in calories leads to increased acetate production by bacteria in the gut. The acetate enters the blood and travels to the brain. As a result, the brain signals to the pancreas to increase insulin secretion and storage of fat, and signals to the stomach to release the hunger hormone ghrelin. This process appears to lead to overfeeding and insulin resistance, creating a feedback loop. Additional research will be necessary to determine whether the same mechanism operates in humans and to identify which bacteria in the gut microbiome contribute to the production of acetate. Nevertheless this study describes a novel link between the gut microbiome, obesity, and metabolic syndrome that could be targeted in the development of therapeutics for obesity and diabetes.

Perry RJ, Peng L, Barry NA, ... Shulman GI. Acetate mediates a microbiome-brain- β -cell axis to promote metabolic syndrome. Nature 534: 213-217, 2016.

Tracking Both the What and the When of the Human Diet: Researchers have developed an innovative smartphone application (“app”) to provide valuable

insights into the content and timing of the human diet, and showed that many adults eat over a span of 15 hours or more each day; through a very small pilot study, they have also begun to explore whether limiting the hours of daily eating and drinking may help achieve weight loss. Measuring just what people normally eat and drink during their daily lives is surprisingly difficult. Standard approaches, such as surveys or food diaries, depend on accurate recollection and measurement by study participants, and can be a significant burden, particularly if a person wishes to indulge in between-meal snacks. Further, research suggests that *when* people eat—not just what—may have a significant impact on metabolic health: shift workers have a higher burden of obesity and diabetes; and in animals, 24-hour access to food promotes poorer metabolic health. To get a clearer idea both of what people eat and when, the new study took advantage of smartphone technology. Participants—156 healthy adult men and women—were asked to use the cameras on their phones to take pictures of everything they ate or drank, regardless of calorie content (including water), for 3 weeks. None of the participants was a shift-worker. An app specially designed for the study logged the date and time of each picture, and sent the image to study researchers for analysis. The image was then automatically deleted from the smartphone to prevent it from later influencing the participant’s eating, and to reduce memory-hogging on his or her device. An estimated caloric value of each item was recorded by study staff. (Participants who forgot to take a picture before they took a bite were asked to submit information *via* text entry.) If participants did not finish an item, they submitted a second picture showing the leftovers. This part of the study was designed to shed light on how people eat: the participants received no dietary guidance.

Results showed that although the majority of participants described themselves as three-meal-a-day eaters, the actual number of times people consumed calories varied substantially, averaging from a little more than 3 to a little more than 10. The median portion of the day during which people ate was nearly 15 hours (*i.e.*, one half of participants generally ate during a shorter period of the day than that, and one half during a longer one). The study also found that consumption was generally heaviest in the evening hours: less than one-quarter of calories were typically consumed before noon, while over one-third were

typically consumed after 6 p.m. The average weight of the subjects remained quite stable over the course of the study; this suggests the simple act of taking the pictures and using the app did not induce participants to make significant changes in their eating habits. However, there are some important caveats: the study was relatively short, and the participants relatively young (average age about 28) and not especially diverse—more than three-fourths of the group were either non-Hispanic white or of Asian descent. Thus, it will be important to learn whether the observed eating patterns are similar to those that would be found in a more representative cohort of Americans.

Because experiments in animal models have shown that reducing food availability to 12 hours or less may have metabolic benefits, the researchers sought to test whether this might work in people. They began with a very preliminary study of just a few people, to see whether such a test would be feasible. They recruited eight participants (five men, three women) from the first study who consumed calories for 14 hours or more per day and who were also overweight or obese to participate in a 16-week follow-up study. These eight people were asked to confine consumption of calories to a consistent 10- to 12-hour window that they themselves were allowed to select. They were instructed to stick to their chosen window, but given no guidance about what kinds of things they should eat and drink, or how much. All eight significantly reduced their eating period—by an average of more than 4.5 hours. Although they were not counseled to eat less, they also consumed 20 percent fewer calories, on average, and most of the people lost several pounds. At the same time, they reported feeling they had more energy, reduced hunger at bedtime, and better sleep satisfaction. Notably, all of the participants expressed interest in continuing this approach, and all of these improvements generally persisted for at least a year, 8 months after the active phase of the intervention had ended. Follow-up studies with larger numbers of people will be needed to confirm these preliminary findings, and to see whether this sort of intervention might be effective in a more diverse group of people. If so, shortening the period of daily caloric intake may turn out to be a valuable approach to helping people lose weight and potentially improve other aspects of their health. In the publication of their study findings, the researchers noted that they are continuing to gather data using the app, and provided information for people

interested in their research to learn more and, if they wish, sign up to participate.

Gill S and Panda S. A smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits.

Cell Metab 22: 789-798, 2015.

BARIATRIC SURGERY RESEARCH

Weight Loss and Health Benefits from Bariatric Surgery in Teens with Severe Obesity: In a study of teens with severe obesity, bariatric surgery resulted in substantial weight loss and improvements in health and quality of life 3 years after the surgeries were performed; the study also identified risks associated with the surgeries. These findings are from the Teen Longitudinal Assessment of Bariatric Surgery, or Teen-LABS, study.

Obesity increases risk for type 2 diabetes, cardiovascular disease, and many other serious conditions. Previous research has shown that adults with severe obesity (also known as extreme obesity) can experience dramatic health benefits from bariatric surgery. However, very little has been known about the effects of this surgery in adolescents, particularly over the long-term—even though it is used in clinical practice for this age group. Thus, researchers designed Teen-LABS, an observational study that enrolled adolescents who were already planning to have bariatric surgery. Their goal was to collect outcome data on health risks and benefits that could help with treatment decisions.

Conducted at five U.S. clinical centers, Teen-LABS enrolled 242 people ages 13-19. Prior to surgery, all were obese, and nearly all had severe obesity, based on body mass index (BMI), a measure of weight relative to height. The majority of the participants in the study were Caucasian females, a demographic representative of patients who seek bariatric surgery at these clinical centers. The study focused on those who underwent either of two bariatric surgical procedures: gastric bypass (used for a majority of the teens), or sleeve gastrectomy. Before surgery, the participants' average weight was 328 pounds. Three years after surgery, their weight decreased by an average of 90 pounds, or 27 percent. Some of the participants had type 2 diabetes, some had

kidney disease, and many had high blood pressure or abnormal levels of blood lipids (cholesterol or triglycerides) prior to surgery. The study found that 95 percent of the teens who had type 2 diabetes had reversal of their disease, 86 percent of those with kidney damage experienced improvements in kidney function, and most of the teens with high blood pressure or lipid abnormalities saw improvements in these conditions 3 years after surgery. Additionally, 26 percent of the teens were no longer obese 3 years after surgery. Although a majority still had some level of obesity, not as many had severe obesity.

The study also identified risks. During the study period, 13 percent of participants needed additional abdominal surgery, most commonly gallbladder removal. The study also found that although fewer than 5 percent of the teens were iron-deficient before surgery, more than half had low iron stores 3 years later.

These results contribute important knowledge about the benefits and risks of bariatric surgery in adolescents. However, further research will be critical to determine the longer-term effects of bariatric surgery on health and well-being, including whether health improvements are sustained and whether additional risks emerge. This information will help teens, their parents, and their health care providers make more informed treatment decisions, so that young people with obesity can have improved health during adolescence and as they become adults.

Inge TH, Courcoulas AP, Jenkins TM,... Buncher CR; for the Teen-LABS Consortium. Weight loss and health status 3 years after bariatric surgery in adolescents. [N Engl J Med](#) 374:113-123, 2016.

Weight-loss Surgery Contributes to Type 2 Diabetes Remission: A study has shown that one type of weight-loss surgery is more effective than another at inducing long-term type 2 diabetes remission in people who have obesity. When approaches to weight loss such as diet, exercise, and medications are ineffective in inducing enough weight loss to produce health benefits, some people with severe obesity turn to surgical options for weight loss—so-called bariatric surgery. While bariatric surgery can be a useful tool to promote and sustain substantial weight loss, increasing evidence suggests it can also be beneficial in treating diabetes. Because type 2 diabetes is associated with excess weight and is a major public health concern,

researchers set out to understand better the effects of two different types of bariatric surgery, Roux-en-Y gastric bypass (RYGBP) and laparoscopic gastric banding (LAGB), on diabetes remission using the large observational study, Longitudinal Assessment of Bariatric Surgery-2 (LABS-2).

Many of the LABS-2 study participants had type 2 diabetes prior to surgery; of these individuals, 466 underwent RYGBP and 140 underwent LAGB. When the team examined diabetes remission rates in LABS-2 participants who underwent RYGBP versus those who had LAGB, they found that, after 3 years, both types of surgery resulted in a subset of participants in each group entering diabetes remission. In other words, both procedures were effective to an extent, such that 68.7 percent of the RYGBP participants, and 30.2 percent of the LAGB participants had blood glucose (sugar) levels that were no longer in the range of diabetes, and they did not need diabetes medications. They also found that the greater the post-surgical weight loss, the greater the chances of diabetes remission after both procedures. Not surprisingly, they observed that individuals in both groups who had better blood glucose control prior to surgery had better remission outcomes. However, when they analyzed changes in certain hormones that typically coincide with weight loss, such as a reduction of overall leptin levels and improved insulin sensitivity, they found something unexpected—these metabolic markers were only associated with diabetes remission after LAGB, not RYGBP. When they examined the relationship between weight loss and diabetes remission more carefully by accounting for weight-loss differences between the two groups, they found a nearly twice as high remission rate for individuals who underwent RYGBP. In other words, weight loss was not the only factor contributing toward diabetes remission after RYGBP. This suggests that RYGBP may have added benefits beyond weight loss on glucose control.

This study found that factors in addition to weight loss may play a role in the greater likelihood of diabetes remission following RYGBP compared to LAGB. Longer-term studies are needed to confirm this.

Purnell JQ, Selzer F, Wahed AS,... Wolfe BM. Type 2 diabetes remission rates after laparoscopic gastric bypass and gastric banding: results of the Longitudinal Assessment of Bariatric Surgery Study. [Diabetes Care](#) 39: 1101-1107, 2016.

Obesity Research Workshops

Obesity—a condition affected by many different factors, including genetics, lifestyle, and even various types of gut bacteria—has soared to epidemic levels in the United States. With this in mind, the NIDDK sponsored two workshops in December 2015 to further explore different aspects of obesity.

Understanding Behavioral Traits Linked to Differences Among Individuals in Physical Activity and Sedentary Behavior

The health benefits of regular exercise and reduced sedentary time are well established. Moreover, it is widely acknowledged that physical activity is an integral part of preventing obesity and maintaining weight post weight-loss. While significant health-promotion efforts have been made throughout the years, substantial room remains for increasing physical activity and reducing sedentary behavior for weight management. To understand behaviors related to variation in physical activity better and to identify promising research opportunities, the first workshop, entitled “Behavioral Phenotyping of Physical Activity and Sedentary Behavior,” was held December 1-2.

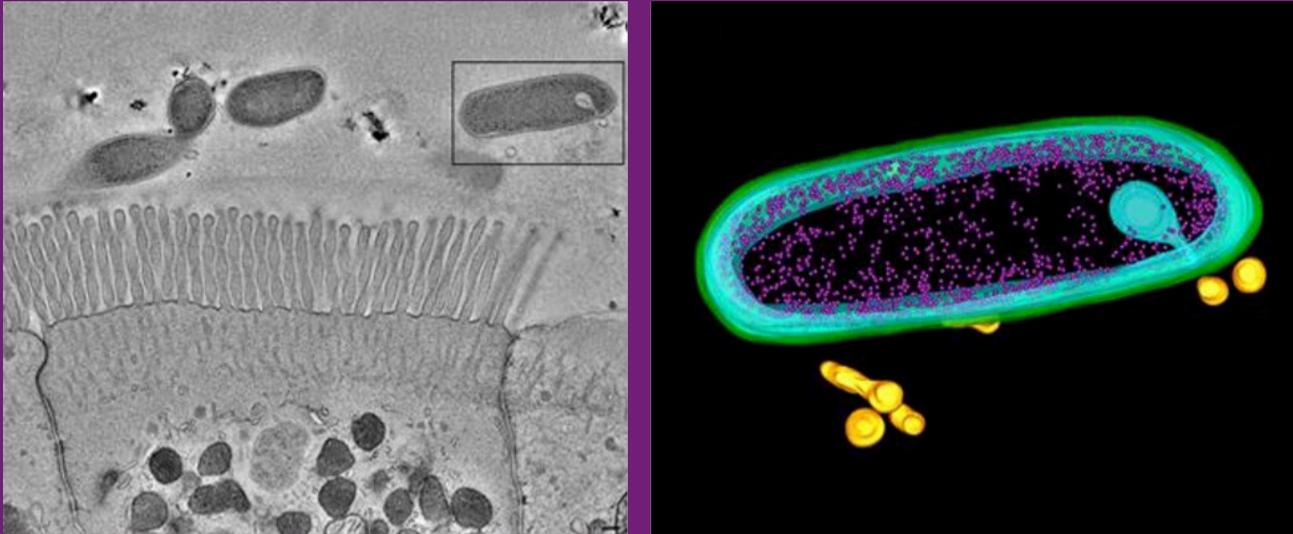
The meeting's objective was to enhance understanding of and identify research gaps in behavioral and psychological factors that influence individual variation in physical activity and sedentary behavior across the lifespan. Speakers summarized the state of the science and identified numerous research gaps. While some promising phenotypes to explain individual variability were discussed, it

was clear that more research is needed. A report is currently being developed to describe the rationale and outcomes of the workshop.

Obesity and the Bacteria and Other Microbes That Live in the Gut (the Gut Microbiome)

Energy balance is the relationship between “energy in”—food intake—and “energy out”—calories burned. Increasing evidence suggests that gut microbial composition—or the gut microbiome—may alter this balance and contribute to the development of obesity. Recent data indicate that the gut microbiome can predict body composition (lean or obese) with 90 percent accuracy compared to 60 percent accuracy with genetics alone. Moreover, evidence suggests the microbiome plays a critical role in weight loss interventions, but the precise functional nature of this role has not been clearly established.

To address unmet needs in this area of obesity research, the NIDDK together with the National Heart, Lung, and Blood Institute, the National Cancer Institute, and the Office of Dietary Supplements organized a second workshop, entitled “Functional Role of Microbiome in Obesity,” held on December 14-15. The meeting brought together experts in microbiome and obesity research, NIH staff, and selected trainees doing research in these areas. A number of research gaps were identified and specific recommendations for potential future research directions were made. Proceedings of the workshop will be published in a scientific journal.



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

Images provided by Dr. Sarkis Mazmanian, California Institute of Technology.
Credit: Mark Ladinsky/Greg Donaldson/Caltech

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple big data sources estimated that digestive disease is the primary diagnosis in a total of 72 million ambulatory care visits to physicians' offices and hospital emergency and outpatient departments in the United States each year.¹ In addition, an estimated 4.6 million hospitalizations with a primary diagnosis of digestive diseases and 13.5 million hospitalizations with a primary or secondary diagnosis of digestive diseases are reported.¹ More recently, a study focusing specifically on the clinical and economic burden of emergency department visits reported 15.1 million emergency department visits with a primary diagnosis of digestive diseases and 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 determine how widespread these diseases are across the United States and in specific population groups, to identify their causes and how they progress, and to test new interventions for prevention and treatment, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by damaging inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment frequently requires prolonged use of multiple drugs and may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and other factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair

damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

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

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

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

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

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

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

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis and their

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

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

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are

identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss, obesity-associated disease, and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

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

GUT MICROBES IN HEALTH AND DISEASE

Nourishing Gut Bacteria To Help Young Children

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

For the first study, the group of scientists began by analyzing fecal samples collected for a previous study

of young twins and triplets, ranging from newborns to 3-year-olds, living in rural parts of another country, Malawi, to determine whether a particular set of gut bacterial species was associated with age-appropriate growth in healthy children or with reduced growth in undernourished ones. Similar to the earlier study by the group in Bangladesh, they discovered that undernourished children had a more immature community of gut microbes than their healthy counterparts. Also, the level of gut microbial maturity at 12 months of age predicted growth at 18 months. To see whether this abnormal set of gut microbes may be contributing to growth and health problems, they looked at effects in an animal model. They transplanted gut microbes taken from 6- and 18-month-old healthy or undernourished Malawian children into young male mice raised under sterile conditions free of any microbes (“germ-free”) and fed a typical Malawian diet. They showed that the immature microbial community from undernourished children can impair growth in mice, based on measurements of lean body mass gain and bone morphology, as well as altered liver, muscle, and brain metabolism. When housed in the same cage, mice that received microbes from healthy infants were able to transmit them to other mice that had received microbes from undernourished children, thereby preventing their growth impairment. They also identified two particular strains of bacteria from the healthy infants that had helped the transplanted mice thrive. Adding these two bacterial strains to the bacteria from undernourished children before giving the mixture to mice also prevented growth impairment.

In another study, members of the same research group, together with other colleagues, analyzed breast milk samples from Malawian mothers with healthy or undernourished infants 6 months after birth to identify nutrients that might interact with the children’s gut microbes and affect their susceptibility to undernutrition. They found that sugars called sialylated oligosaccharides—which are digested not by humans, but by their gut bacteria—were less abundant in the breast milk of mothers with severely growth-stunted infants. They then tested whether sialylated oligosaccharides from cow’s milk could promote growth, using a mouse model. After transplanting fecal microbes from one of the 6-month-old stunted infants into young male germ-free mice, they fed the mice a typical Malawian diet, either with or without added sialylated oligosaccharides. They showed that mice that received

the supplemental sialylated oligosaccharides had greater muscle mass and positive changes in bone morphology and liver, muscle, and brain metabolism. These changes indicated an improved capacity for utilizing nutrients and gaining weight, despite the context of a deficient diet and gut bacteria associated with stunting. Mice that were kept germ-free while receiving the diet with sialylated oligosaccharides did not show the same beneficial effects of these sugars. These results were also verified in another animal model, the germ-free piglet, which has a physiology closer to that of humans. Results in these animal models suggest that consumption of milk high in sialylated oligosaccharides affects microbes in the gut, which in turn may promote growth in undernourished children consuming a nutrient-deficient diet.

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

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Early-life Exposures Affect Infant Health: Three recent studies have shown how dietary and other environmental exposures, including those that shape the internal environment created by gut microbes, are critically important during the first few years of life, with implications for a lifetime of good health. These exposures include not only the diet of the mother and child, but also other experiences that have a large impact on the bacterial populations of a child's gut, such as antibiotic treatment and delivery by vaginal or cesarean modes. More and more, the gut microbial community is being appreciated for its effects on human health, and the first 3 years of life is an important period for maturation of this gut microbial

community. For example, by training the developing immune system, gut microbes are thought to play a possible role in guarding against autoimmune diseases such as type 1 diabetes and inflammatory bowel disease, as well as other immune-related diseases, including asthma and allergies. Early disturbances in the gut microbial community from such factors as antibiotics or cesarean delivery have also been linked to an increased risk for metabolic disorders, such as obesity. Studies by three research groups have delved into how great an impact these early exposures can have on infants, potentially affecting their future health.

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

Another research group followed the gut microbial development of 43 U.S. children during their first 2 years using genetic techniques to characterize the evolving community of bacterial species present in their stool samples during this dynamic period of development. They collected vaginal swabs, rectal swabs, and stool samples from mothers, both before and after delivery, and stool samples from the infants. Typically, infants'

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

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

less stable gut microbial community and an increase in antibiotic resistance genes.

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

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Eating Fiber for the Health of Future Generations:

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

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

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

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

Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, and Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature* 529: 212-215, 2016.

Understanding How Crohn's Disease Treatments

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

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

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

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

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GENETIC UNDERPINNINGS OF INFLAMMATORY BOWEL DISEASE

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

variations that are present in people with IBD. Studies over the past several years had identified 163 areas of the genome that are linked to IBD, most of which are shared between Crohn's disease and ulcerative colitis.

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

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

magnitudes of effects. This information could enable care providers in the future to tailor treatments based in part on the patient's genetic background.

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

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

One of the research groups concentrated on the genes *ATG16L1* and *NOD2*, both of which code for proteins that are known to play important roles in autophagy and have variants that are implicated in IBD. The scientists found that immune cells from

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

Another team of scientists investigated the role of autophagy as a cellular defense mechanism against potentially harmful bacteria. Some types of bacteria can invade cells, causing disease, and cells typically use autophagy to package and degrade the invading microbes. Armed with this knowledge, the researchers performed genetic screening in a human cell line to identify genes implicated in IBD that are involved in both autophagy and cellular defense against bacteria. Among the genes they identified was *GPR65*, which has variants associated with IBD. *GPR65* encodes a protein that is important for the proper function of lysosomes, which are acid-rich globules in cells that break down material packaged for autophagy. The researchers found that male and female mice without functional *GPR65* protein were more prone to a disease resembling human IBD when given a type of bacteria that causes intestinal inflammation in mice. This effect was

seen when GPR65 was absent from either the cells lining the gut or the immune cells within the gut. The lysosomes of intestinal and immune cells lacking GPR65 were unable to properly degrade invading bacteria. This could be explained by the observation that the lysosomes were not positioned properly in the cell and were not as acidic as normal lysosomes. Importantly, the researchers also tested a human cell line engineered to have a genetic variant found in male and female IBD patients, and immune cells from IBD patients who have this variant, and they found that these cells were also defective in destroying invading bacteria. These results suggest that this genetic variant of *GPR65* could promote IBD by crippling autophagy and cellular defense against disease-causing bacteria.

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

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The Inflammatory Bowel Disease Genetics Consortium



Inflammatory bowel disease (IBD) is the collective term for a group of debilitating digestive disorders, including Crohn's disease

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

The NIDDK's IBD Genetics Consortium (IBDGC) was established in 2002 to identify genes that are involved in IBD susceptibility. In collaboration with the International IBD Genetics Consortium, of which it is a member, the IBDGC has enrolled thousands of IBD patients and identified about 200 regions of the human genome that are associated with risk

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

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

PANCREATITIS RESEARCH

Chronic Pancreatitis: Cause, Not Gender, Determines the Disease:

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

To investigate the role of gender in pancreatitis, researchers analyzed data from 521 men and women who participated in the NIDDK-sponsored North American Pancreatitis Study 2 (NAPS2). All participants had been diagnosed with chronic pancreatitis, and 45 percent of the individuals studied were women, a surprising number for what has historically been thought of as a male-dominant disease. Equal proportions of men and women reported having abdominal pain, and the use of pain medications was similar between men and women. The conditions that commonly accompany chronic pancreatitis, such as pancreatic duct stones and defects in the production of pancreatic enzymes, were also similar between men and women. However, the researchers found that alcohol was more likely to be diagnosed as the cause of pancreatitis in men than women (about 60 percent of men in the study, versus 30 percent of women). In contrast, women were more likely to have pancreatitis due to an obstruction (such as the presence of gallstones) or unknown cause. Women were also more likely than men to undergo

gallbladder removal or sphincterotomy procedure, in which the circular muscles constricting the ducts draining the pancreas are severed to allow the flow of digestive juices. However, the researchers found that these procedures were more likely to be performed in women because the cause of the pancreatitis was unknown, and not because of gender differences in their diseases *per se*. These results suggest that it is the cause of the chronic pancreatitis—such as heavy alcohol use, gallstones, or an unknown cause—and not necessarily the patient's gender that determines how the disease presents itself and how it is best treated. These results could help health care providers determine which patients would benefit from certain courses of treatment, including identifying any biases that may exist in the current forms of treatment chosen for patients of different genders.

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Identifying Risk Factors for Pancreatitis in Children:

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

The multinational INSPPIRE (International Study Group of Pediatric Pancreatitis: In Search for a Cure) consortium was established to investigate the risk factors and outcomes of pediatric pancreatitis. The consortium, which has enrolled the largest cohort of pediatric pancreatitis patients to date, collected genetic, demographic, and clinical data from 301 children (girls and boys aged 19 and under) with acute recurrent or chronic forms of pancreatitis. The most common risk factor for pancreatitis in children

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

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

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IRRITABLE BOWEL SYNDROME RESEARCH

Pain Expectations: Altered Brain Responses in People with Irritable Bowel Syndrome: A recent study suggests that people with irritable bowel syndrome (IBS) engage

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

The new study investigated whether people with IBS show altered brain activity when uncertain about a future abdominal pain experience. The pain experience used in the study was an electrical stimulation delivered via patch electrodes on the abdomen. Prior to the experiments, the amount of stimulation to deliver to each individual was carefully tested to achieve a level that person considered unpleasant but tolerable. Using imaging technology, researchers then looked at the brains of men and women with or without IBS under several repetitions of three conditions: when told verbally and with a visual cue that they may expect an unpleasant external stimulus to the abdomen within a certain time frame (“cued threat”); when told verbally and with a visual cue that there would be no stimulus within a certain time frame (“cued safe”); and when told verbally that there would be no stimulus, but without a visual cue about either the stimulus or the time frame (“uncued”). The experiments were designed to increase the sense of ambiguity and the likelihood that anxiety would be heightened during the “uncued” condition, by alternating the “uncued” and “cued” conditions and leaving the electrodes attached to the abdomen at all times. The scientists found that all participants showed activation of several brain regions in response to both the “cued threat” and the “uncued” condition when compared to the “cued safe” condition. Compared to healthy people, however, people with IBS showed greater activity in brain regions involved in threat appraisal, emotional arousal, and self-consciousness during the “uncued” condition versus the “cued safe” condition. This difference was primarily seen among women with IBS compared to their

healthy counterparts. Ambiguous situations generally lead the brain to engage in developing predictive responses, especially in those with anxiety, which occurs commonly in people with IBS. These results provide clues into the role of brain response to context—*i.e.*, uncertainty—in symptom experience, including symptom hypervigilance, in those with IBS. Future studies should help to refine these findings and flesh out the additional influence of sex and gender.

Hong JY, Naliboff B, Labus JS,...Mayer EA. Altered brain responses in subjects with irritable bowel syndrome during cued and uncued pain expectation. *Neurogastroenterol Motil* 28: 127-138, 2016.

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

Working with male and female rats, scientists have mapped brain changes brought on by stress early in life that appear to heighten responses to uncomfortable sensations in the abdomen. In these experiments, a condition of early-life stress (ELS) was created by limiting the bedding available to rat pups and their mothers for a week soon after birth. (They were then given normal bedding again.) For comparison, other rat pups were given only normal bedding, so they would not be stressed. At age 10 to 11 weeks, all of the rats were exposed to an uncomfortable sensation in the abdomen, during which both abdominal and brain responses were measured and evaluated using a number of techniques. The scientists found that, compared to the unstressed rats, ELS rats displayed heightened sensitivity in their abdominal responses to the uncomfortable stimulus. Also, while all the rats showed changes in brain activity in response to the stimulus, ELS rats displayed multiple differences from

non-stressed rats in the activation of brain regions involved in pain. Furthermore, ELS rats showed an increase in functional connections between regions of the brain comprising the “pain circuit.” Finally, although male and female rats showed no differences in their abdominal responses to the uncomfortable stimulus, the researchers found sex-based differences in brain activation responses in each group. They also determined that ELS exposure affected male and female brain responses differently.

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

Holschneider DP, Guo Y, Mayer EA, and Wang Z. Early life stress elicits visceral hyperalgesia and functional reorganization of pain circuits in adult rats. *Neurobiol Stress* 3: 8-22, 2016.

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

To examine the link between exposure to early childhood trauma and IBS, researchers administered the ACE questionnaire to a group of 148 people with IBS as well as a similar number of healthy individuals. Study participants included both women and men, although women predominated, particularly in the IBS group. An ACE score was then generated based upon

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

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

Park SH, Videlock EJ, Shih W,...Chang L. Adverse childhood experiences are associated with irritable bowel syndrome and gastrointestinal symptom severity. Neurogastroenterol Motil 28: 1252-1260, 2016.

INSIGHTS INTO ACUTE LIVER FAILURE OUTCOMES

Improved Outcomes and Survival Following Acute Liver Failure in Recent Years: Results from a national, multi-center study spanning 16 years showed that outcomes and survival have improved for people who experience acute liver failure, including those who

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

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

Reuben A, Tillman H, Fontana RJ,...Lee WM. Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. Ann Intern Med 164: 724-732, 2016.

UNDERSTANDING AND TREATING LIVER DISEASE

Toxin Provides Clues to Disease Processes Underlying

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

In one study, the zebrafish larvae model was used to define the biochemical pathways by which biliatresone causes bile duct toxicity. By profiling which genes were turned on in the bile ducts and livers of larvae exposed to biliatresone, they found that genes involved in protecting cells against stress, such as that caused by oxidative damage, were among those most activated as a defense mechanism against the toxin. Most striking after exposure to biliatresone were changes in genes governing metabolism of glutathione, a substance made up of three amino acids that serves as a major antioxidant in cells, specifically responsible for neutralizing toxins from outside the body. Measurements of glutathione levels in the larval cells showed that biliatresone caused a depletion in this important antioxidant, particularly in the bile ducts. Furthermore, when glutathione levels were depleted

with either a chemical or genetic modification to the larvae, the bile duct cells were then even more sensitive to injury by biliatresone. Conversely, the larvae's bile duct cells were more resistant to biliatresone injury when glutathione levels were replenished. A common strategy for boosting glutathione is the use of drug called N-acetylcysteine, which is a glutathione precursor. A second strategy is use of a chemical called sulforaphane, found in vegetables such as broccoli, that activates a master regulator of glutathione synthesis. Both of these strategies led to a decrease in biliatresone toxicity to the zebrafish bile duct cells. These findings point to glutathione depletion as playing a key role in bile duct injury from this toxin. Importantly, they also point to possible means of prevention or control of biliary atresia, such as supplementing the diets of pregnant women with glutathione precursors.

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

Although there is currently no evidence that biliatresone is the specific cause of human biliary atresia, the mechanism by which it injures bile ducts is likely similar to what occurs in humans. These studies also suggest how environmental toxins may play a role early in the disease process. Most importantly, these findings may aid in the development of new treatments or prevention strategies, either in avoiding the environmental toxins that might have similar

effects in pregnant women or in improving antioxidant status and resistance to these toxins by means of dietary supplementation.

Zhao X, Lorent K, Wilkins BJ,...Pack M. Glutathione antioxidant pathway activity and reserve determine toxicity and specificity of the biliary toxin biliatresone in zebrafish. *Hepatology* 64: 894-907, 2016.

Waisbourd-Zinman O, Koh H, Tsai S,...Wells RG. The toxin biliatresone causes mouse extrahepatic cholangiocyte damage and fibrosis through decreased glutathione and SOX17. *Hepatology* 64: 880-893, 2016.

Controlling Levels of Bile Acids for Liver Health:

Researchers have recently revealed an important step in the way the levels of bile acids are regulated in the liver. Bile acids, a component of bile produced in the liver, are critical for digestion and absorption of fats in the small intestine. In addition, bile acids are signaling molecules that affect metabolism. Levels of bile acids must be tightly controlled because excess bile acids can be toxic and lead to cholestasis (reduced bile flow) and liver injury. Conversely, insufficient bile acids can lead to malabsorption and malnutrition. Previous research showed that a protein named “Small Heterodimer Partner (SHP)” plays a key role in regulating bile acid levels, but the details of this process were unknown. In a recent study, scientists discovered a clue to this process when analyzing proteins that bind to SHP when human liver cells were exposed to high levels of bile acid. The most important protein that interacted with SHP was “RanBP2.” Using biochemical and mouse liver cell experiments, the researchers found that, when exposed to high levels of bile acids, RanBP2 chemically modified SHP, facilitating SHP’s movement into the nucleus of the cell. Once in the nucleus, SHP turns off genes that are needed for synthesis and transport of bile acids. In this study, the scientists demonstrated that the chemical modification by RanBP2 was required for SHP’s activity to move into the nucleus and turn off these genes.

To study further the role of SHP, the scientists introduced a version of SHP that could not be chemically modified by RanBP2 into male mice and fed the mice bile acids. They found that bile acid levels increased in the liver, gallbladder, and small intestine of these mice, and observed pathological changes in the liver (liver cell death and increased inflammation). Thus, mice with impaired SHP proteins were unable to decrease bile acid levels to normal. In

a complementary experiment, decreasing the amount of RanBP2 in mice and feeding the mice bile acids led to increased bile acid levels in the liver and gallbladder resulting in an increase in liver toxicity markers and pathological changes in the liver. These experiments indicated that the RanBP2 helps regulate bile acid levels and protects against liver damage. This study illuminated how, upon detecting elevated bile acid levels, SHP acts to reduce the levels. Developing a therapeutic that targets this pathway could be a promising direction for treatment of cholestatic liver diseases and other bile acid-related diseases.

Kim DH, Kwon S, Byun S,...Kemper JK. Critical role of RanBP2-mediated SUMOylation of Small Heterodimer Partner in maintaining bile acid homeostasis. *Nat Commun* 7: 12179, 2016.

INSIGHTS INTO THE DEVELOPMENT OF LIVER CANCER

Key Biomarker Involved in Liver Cancer Development from Multiple Causes:

Scientists have discovered that activation of a protein called p62 in liver tissue already injured by diverse factors plays a central role in promoting the development of the major form of liver cancer, called hepatocellular carcinoma (HCC). HCC is known to develop in response to inflammation and damage caused by a wide range of conditions, including hepatitis B or C viral infections, alcohol abuse, and obesity—specifically the obesity-associated condition nonalcoholic steatohepatitis or “NASH,” a form of fatty liver disease. The scientific community has been searching for a common mechanism by which these diverse causes of liver injury are “precancerous” and can lead to the development of liver cancer. Recently, a group of researchers focused their attention on p62, a protein that participates in transmitting signals inside the cell and that tags damaged proteins inside cells for destruction and recycling. This protein accumulates in many forms of chronic liver disease, including those most closely linked to cancer. The investigators used several mouse models of liver disease, as well as liver samples from patients with HCC. One mouse model involved giving a carcinogenic chemical to animals genetically manipulated to lack p62. The mice that lacked p62 developed fewer liver cancers than those still producing p62. Turning to an animal model of overfeeding that mimics human NASH, the scientists fed a high-fat diet to mice with or without

p62. Progression from fatty liver to HCC was again less in mice without p62 compared to those with normal levels of the protein. To ascertain the actions of p62 in the liver, they next engineered viruses that would infect liver cells in mice and boost their levels of p62. Infection with these viruses boosted p62 levels and caused more liver tumors in mice than did infection with control viruses that did not change the protein levels. The researchers also investigated p62's role in liver cancer progression in humans. Using tissue samples from patients with HCC who had undergone surgery to remove the tumor, they found that patients with high levels of p62 in the

remaining liver had reduced survival, likely due to HCC recurrence. This study suggests that p62 is important in the complex and varied pathways that lead from liver injury to HCC development and suggests this protein may be a promising target for future therapeutics or in providing a valuable biomarker for identifying people with HCC at increased risk of recurrence, even after surgical removal of their tumors.

Umemura A, He F, Taniguchi K,...Karin M. p62, upregulated during preneoplasia, induces hepatocellular carcinogenesis by maintaining survival of stressed HCC-initiating cells. Cancer Cell 29: 935-948, 2016.

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

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

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

Another workshop, entitled "**Chronic Pancreatitis in the 21st Century: Research Challenges and Opportunities,**" was held on July 27 to address new

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

Summaries of these workshops will be published in major scientific journals. The knowledge shared at these workshops will help steer research toward providing new pathways for diagnosis and treatment for these diseases.

Illuminating the Inner World of the Gut Microbiome and Its Impacts on Human Health

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

The Microbial “Organ” Within

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

Microbes found in the gut carry their own genes and perform many important functions that human cells lack, thereby augmenting the body’s genetic and

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

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

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

Scientists have made great strides in elucidating the microbial species present in the human gut and how they make this habitat their home, as a foundation for revealing their contributions to human health and

STORY OF DISCOVERY

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

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

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

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

STORY OF DISCOVERY

What Are They Doing in There?

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

Boosting Digestion and Immune Function

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

Turning to effects of the gut microbiome and host-microbe interactions on properly calibrating host immune function, researchers showed in mice that a gut cell type called a Paneth cell produces a molecule that preferentially attacks harmful, invading microbes. Another group of scientists used advanced genomic tools and data, some swimming bacteria, and see-through zebrafish to track the movements and host impacts of gut bacteria, demonstrating the bacteria's beneficial effects on fish immunity. Scientists also found that conventionally raised mice are able to clear infection by virulent bacteria, but mice raised germ-free are not, suggesting that infection and clearance of intestinal pathogens is the result of virulence factors carried by the invaders and a competition for nutrients with other microbes. Research also showed that intestinal cells can sense

potentially harmful bacteria nearby and release an antimicrobial protein to help create a protective buffer zone between the inner walls of the small intestine and the bacteria contained within. A similar study showed that another protein produced by intestinal cells contributes to creating this buffer zone by selectively puncturing the protective outer membranes of targeted bacteria. Other studies focused on one particularly “friendly” bacterial species, *Bacteroides fragilis*. Scientists found that this species actively engages with gut immune cells through molecular communication processes to maintain the “tolerance” response required for a colonization and an ongoing symbiotic relationship. Using mouse and cell models, researchers uncovered one way in which beneficial microbes in the intestine support healthy immune function in their hosts—by releasing substances called sphingolipids that keep the activity of the local immune cells in check. Recently, scientists explored how different human gut bacterial strains modulate functions such as immunity and metabolism. To do this, they transplanted gut microbes isolated from human stool samples into germ-free mice and found that several bacterial strains are associated with an expansion of immune cells in the colon, as well as an increase in fat stores.

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

STORY OF DISCOVERY

Obesity

Pioneering studies in this field have been particularly prolific in investigating the role of the gut microbiome in obesity. Early studies found that conventionally raised mice have more body fat than their germ-free counterparts; when the germ-free mice were given microbes, they dramatically increased their total body fat, even while decreasing their food consumption. The scientists showed that gut microbes increase the amount of calories harvested from the diet and also boost production of liver enzymes involved in fat production. In other experiments, researchers used a “humanized” mouse model to show that two microbial species in the human gut—*Methanobrevibacter smithii* and *Bacteroides thetaiotamicron*—have a cooperative relationship in digesting fiber that leads to more efficient nutrient absorption and energy storage as fat. Another ground-breaking study was one of the earliest to show a possible role for gut microbes in human disease; it provided evidence that the relative abundances of two types of dominant beneficial bacteria in the gut are altered in obese humans, and that their balance is restored with weight loss. Studies in lean and obese mice and humans revealed how some gut microbes not only contribute to providing extra calories by extracting more energy from food, but also modulate the biologic pathways that regulate metabolism and whether calories are burned or stored as fat. In a study of obese and lean adult twins and their mothers, researchers examined the human gut microbiota through fecal samples to determine factors associated with bacterial composition. The researchers found that obesity was associated with significantly less gut bacterial diversity than leanness. In another study, researchers showed how changes in gut bacteria play a surprising role in the progression of nonalcoholic fatty liver disease, a condition associated with obesity. Researchers revealed that gut microbes from pairs of human twins—one obese and the other lean—can

transmit these body types to mice, making them gain or lose weight, in conjunction with their diets. To gain new insight into a form of gastric bypass surgery, a treatment for obesity, researchers studying a mouse model found that restructuring of the digestive tract leads to weight loss and metabolic benefits in part by altering the communities of bacteria that normally live in the intestines. Research on a large population of twins in the United Kingdom showed that genetic factors shape the composition of the gut microbial community, and that some gut microbes, such as those in the microbial family *Christensenallaceae*, may in turn affect human metabolism and propensity for weight gain. Scientists comparing different breeds of mice discovered that genetics, diet, and gut microbes acquired in different environments all interact to modify susceptibility to obesity and other metabolic conditions, such as insulin resistance.

Malnutrition

On the other end of the nutritional spectrum, malnutrition, particularly in children, has also been an area of intense investigation for gut microbiome researchers. A study in Malawi showed that gut microbes may play an important role in causing a severe acute form of malnutrition called “kwashiorkor” in children that persists in spite of nutritional interventions. A similar study in an impoverished urban area of Bangladesh discovered that children who are malnourished do not harbor gut bacteria typical for their age, but rather display an “immature” gut microbiome, even several months after receiving a nutritional intervention. Through further work in Malawi, researchers identified a group of bacteria in fecal samples from severely undernourished infants and children that take hold in the gut under conditions of nutrient deficiency, thwarting the body’s ability to absorb available nutrients in the diet and to fend off disease. Recently, scientists also analyzed milk samples taken from Malawian mothers with healthy or undernourished infants 6 months after birth to identify nutrients

STORY OF DISCOVERY

called sialylated oligosaccharides that specifically interact with gut microbes and affect their children's susceptibility to malnutrition. Another recent study in Malawi analyzed the immature microbes present in undernourished children; transplanting these microbes into mice, the researchers showed that they impair growth of the mice. Giving these mice two other gut bacterial species, taken from healthy mice, improved their growth. These findings could lead to the development of interventions to modify gut bacteria in undernourished children, to improve the children's growth and health.

Inflammatory Bowel Disease

The human gut microbiome has also been shown to play an important role in inflammatory bowel disease (IBD). An early study in a rat model found that the composition of gut microbes is one factor influencing IBD. Another study showed how short-chain fatty acids produced through gut bacterial fermentation of dietary fiber act on immune cells to protect against intestinal inflammation in mice. One team of scientists discovered how the gut bacterium *Bacteroides fragilis* interacts with the immune system to suppress IBD in mice by releasing a substance called polysaccharide A in small spheres, called outer membrane vesicles, which bud from the bacterial cells' outer coating. Scientists also showed that mice with a pre-existing genetic susceptibility to intestinal inflammation fed a diet high in saturated fats from milk have altered intestinal microbial communities that occur along with changes in bile acid composition, altered immune function, and increased intestinal inflammation. These findings outline a compelling picture of how genetics, immunity, diet, and microbes interact in the development of conditions such as IBD. Other studies used mouse models to detect the relationships of microbes and immune cells to IBD and other immune-related diseases by showing that exposing pregnant mice to "friendly" bacteria shortly before delivery protected their offspring against chemically induced ulcerative

colitis. Researchers also identified disease-related changes in the gut bacterial community of children with IBD, along with changes in gene activity that occurred within their gut cells, resulting in a particular microbial and genetic "signature" that could provide targets for improving diagnosis and therapy. Another group of researchers attempted to identify the bacteria associated with IBD by determining which bacteria are coated with a type of "antibody" or immune protein, called IgA, that the body produces to protect itself from foreign substances. Research on the gut "virome" points to viruses called Caudovirales inhabiting the human gut as other possible culprits in IBD. More recently, a study in children and teens showed that different treatments for Crohn's disease, such as immunosuppressive medication or a defined formula diet, have varying effects on the gut microbiome—a finding with implications for approaches to monitoring treatment response and for potentially developing microbiome-targeted therapies. Two recent studies of the genetics of individuals with IBD have pointed to abnormal interactions between the gut and the bacteria that inhabit it in these cases, implicating genetic defects in a process called autophagy that cells use to break down microbial material.

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

STORY OF DISCOVERY

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

Other Digestive Diseases

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

Ongoing Research Efforts

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

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

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

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

A Fresh Take on Energy Balance Regulation

Severe obesity is a chronic condition that, for many people, is difficult to treat with diet or exercise alone and increases risks for type 2 diabetes, cardiovascular disease, fatty liver disease, and many

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

Bariatric Surgery as a Treatment for Severe Obesity

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

SCIENTIFIC PRESENTATION

used in clinical practice and that induce weight loss but also have metabolic effects independent of weight loss:

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

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

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

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

showed reduction in mortality in people who had bariatric surgery when compared with similar people who did not.

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

Molecular Mechanisms Underlying the Effects of RYGB Surgery

Determining the molecular and cellular changes caused by bariatric surgery requires a robust animal model in which to test hypotheses. Dr. Kaplan's group developed a mouse model of RYGB that could be used for research, taking into consideration the minor differences between mouse and human GI systems. In a typical experiment, these mice were fed a high-fat diet to induce obesity, and divided into three groups. RYGB was performed on one group of mice, and a second group was given a sham operation as a control (referred to as "SHAM" mice). Following a recovery period, both groups were returned to a high-fat diet. SHAM mice returned to their original weights within 2-3 weeks, but RYGB mice maintained an approximately 40 percent reduction in weight for the duration of the 12-week experiment. A third group was given the sham surgery, but underfed following the recovery period (referred to as the weight-matched sham, or "WMS" group), resulting in body weights that matched those

SCIENTIFIC PRESENTATION

in the RYGB group. Comparisons between the RYGB and WMS groups can help distinguish the specific physiological effects of bariatric surgery from those that occur as a result of weight loss alone.

Deeper analysis of these mice revealed that RYGB mice lost weight, in part, because of increased energy expenditure, not simply by eating fewer calories. But what is causing the increased energy expenditure that was observed? Dr. Kaplan is currently exploring some possible explanations. For example, his group has evidence suggesting that RYGB is activating brown and beige adipose tissues—two distinct types of fat that burn calories to generate heat.

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

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

mutations that rendered both copies of their MC4R non-functional.

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

Interactions Between the Gut Lumen and the Body

Although bariatric surgery procedures differ in the way they remodel the GI tract, Dr. Kaplan pointed out that one common feature is dramatic, albeit different, changes to the composition of the contents within the lumen at every level of the gut. These contents, which include the microbiome, enzymes,

SCIENTIFIC PRESENTATION

and bile acids, varied in different regions of the GI tract among mice that had different procedures. (Bile acids are chemicals released from the gallbladder into the upper portion of the small intestine, normally aiding in the digestion and absorption of nutrients; they also act as hormones, influencing metabolism and other physiological processes.) Research from Dr. Kaplan and other scientists has revealed enormous complexity in the interactions between these luminal contents and a variety of functions of the body, such as nutrient absorption, heat production, appetite, circadian rhythms, and pancreatic function. Bariatric surgery disrupts these interactions, leading to the observed physiological changes.

Conclusions

Hypotheses explaining the mechanisms behind bariatric surgery's effects have evolved over the years. Initially, bariatric surgery was thought to work simply through the mechanical restriction of the GI tract to reduce the amount of food that the body could ingest or absorb. Over time, the models became considerably more complex,

including physiological changes caused by bariatric surgery, and later adding the integration of specific molecular "chokepoints" (e.g., MC4R) that allow for blocking the procedures' effects (e.g., with the MC4R mutations in mice). Dr. Kaplan suggested that global influences of bariatric surgery, as illustrated by the vast number of genes, proteins, and metabolites affected by these procedures, drive highly integrated metabolic changes throughout the body. In the early phase of this work, he anticipated that pharmacological treatment targeting a small group of pathways might be able to reproduce the effects of bariatric surgery, and his focus was on identifying those critical pathways. The sheer complexity of the physiological response to bariatric surgery has changed his perspective. Based on these more recent studies, he noted that the ideal targets of "surgicomimetic" therapy likely reside in the gut itself. He concluded that better understanding of the local GI signals that induce these complex, global physiological effects of bariatric surgery will likely provide the fastest route to new and more effective treatments for obesity, diabetes, fatty liver disease, and related metabolic disorders.

One Man's Experience Surviving Acute Liver Failure

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

From Aches and Pains to Organ Failure in 24 Hours

In late February of 2016, Scott was feeling ill with fever, muscle aches, and nausea. Staying home sick in Ruckersville, Virginia all week from his job as a production art manager at a T-shirt design company in nearby

Charlottesville, he started to feel better, then took a turn for the worse on Sunday night. "Every single joint ached. It hurt to move," he remembers. With a high fever that caused him to sweat through his bathrobe and sheets, and nausea limiting his water intake, he became profoundly weak. "I was walking in my kitchen, trying to make some tea, and had to put myself on the floor rather gently," he recalls. After losing consciousness, he later woke up and remembers thinking "I've got to get back to bed." When the local sheriff came by at the request of his company to check up on him, he was able to answer the door and agreed to have an ambulance called. "I just wasn't feeling right.... I just figured I had the flu," says Scott.

"Every single joint ached.... I just figured I had the flu," says Scott. But tests of his liver and kidney functions came back with alarming results.

At the local outpatient emergency room on Sunday, February 28th, the staff treated his dehydration with intravenous saline. Scott called his workplace to let them know where he was and that he would likely be out for a few more days. As he understood it, the treatment plan was "...we're going to get some fluids in you, we're going to get you kind of stable there, and then we're going to see if you can keep fluids down ... if you can do that, we'll send you home." But over the next day and into early Monday, it became clear that something more serious was going on. Tests of his liver and kidney functions came back with alarming results: possible kidney and liver failure. He recalls the doctors telling him, "You look fine, but your numbers say you are not fine.... You are going to be sent to the hospital." The staff quickly called around to area hospitals to see which intensive care units had beds available.

He was ultimately transferred an hour's drive away to Virginia Commonwealth University (VCU) in

Richmond. In the early hours of Monday, February 29th, Scott was driven by ambulance to VCU, feeling awful and vomiting blood. "It was a pretty long drive," he recalls.

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

PATIENT PROFILE

potentially harmful effects on the liver by friends who were doctors. Instead, he had taken only aspirin for pain relief during the past week. They also asked him routine questions to test his mental clarity, considering that his organ failure had elevated ammonia levels in his body to a point that can cause cognitive impairment and even coma. "They kept asking, 'What day is it? Who's the President?'" Scott recalls. Despite his discomfort and precarious situation, he retained his sense of humor. When asked once again by his doctors if he took drugs, he waved his hand attached to the intravenous (IV) drip, saying, "You mean this stuff?"

The medical team continued to search for clues to the cause of his organ failure. The sudden onset

and marked abnormalities in his liver test results would

usually point to an

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

"You look fine, but your numbers say you are not fine.... You are going to be sent to the hospital," Scott recalls his doctors saying.

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

Acute Liver Failure and Reye Syndrome

Acute liver failure or "ALF" is relatively rare in the United States, but can be caused by over-the-counter and prescription drugs, dietary supplements, and herbal remedies. Its most common cause in this country is the over-the-counter pain reliever acetaminophen.

However, in Scott's case, he had avoided taking acetaminophen during his illness,

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

PATIENT PROFILE

Reye syndrome is marked by dysfunction in the mitochondria—structures within cells that generate their energy—causing a build-up of fat in the liver and lactic acid in the blood. This likely occurred in Scott when he took aspirin during his infection with a flu-like virus. Along with the acute liver failure of Reye syndrome, ammonia levels rise in the blood and enter the brain, where they can cause swelling, as well as confusion, altered consciousness, and even coma. The syndrome also causes depletion of another energy source within the liver: glycogen, a stored form of glucose, as the body tries to compensate for the failing mitochondria. If appropriate medical care is not received, the syndrome can swiftly turn fatal. Fortunately, the effects of Reye syndrome can reverse spontaneously

once aspirin is stopped. Its negative consequences can be managed by

supporting the patient during the dangerous period of severe liver and kidney failure, so that the injury is not permanent and the organs recover with time.

STOPping Acute Liver Failure in Its Tracks

In Scott's case, his doctors attributed his acute liver failure to an adult form of Reye syndrome, caused by his use of aspirin in combination with his flu-like viral infection. After consenting to participate in the clinical trial, called "STOP-ALF," he was treated over the next few days with an experimental drug called ornithine phenylacetate, delivered intravenously, in addition to the other standard medical treatments he received. Based on promising results from prior research, the doctors hoped that the drug would detoxify the ammonia buildup caused by his failing liver and thereby protect his brain while the liver and kidneys slowly recovered. The doctors continued to monitor his liver and kidney functions, which soon started to improve, obviating any further discussion of a transplant. His mother stayed nearby and acted as

an advocate for Scott, taking notes during visits from the doctors and handling calls to his health insurance company. During his second week in the hospital, his liver function numbers were back to normal, while the kidneys took some additional time to recover. By the time he left the hospital on March 15th, his liver had fully recovered, but he was scheduled to come back for more dialysis. Fortunately, at that later appointment, he was informed that his kidneys had improved to the point where dialysis was no longer required. He has continued to return to VCU to check in with his doctors there.

The STOP-ALF trial is part of a larger research effort supported by the NIDDK called the Acute Liver Failure Study Group, a group of clinical centers

throughout the country committed to advancing understanding of acute liver

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

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

"If it helps other people," Scott says of his participation in the STOP-ALF clinical trial, "I'm more than happy to participate."

PATIENT PROFILE

injury caused by drugs and other agents. The NIDDK also partners with the NIH's Library of Medicine on the "LiverTox" website (<http://livertox.nih.gov/>), which features sample cases of people with drug-induced liver injury based on the Network's data, as well as a database summarizing liver injuries caused by drugs, including aspirin, acetaminophen, and various herbal and dietary supplements.

Life After Surviving Acute Liver Failure

All signs indicate that Scott has made a full recovery from his liver and kidney injury. In mid-April 2016, he was able to return to work and conquer the "tsunami" of email received during his absence. He has also been

able to get back to enjoying his hobbies, including building model cars and seeking real project cars

to work on. However, his stamina remains limited, as he notices in his regular activities, such as when he uses his push lawnmower on his half-acre property. Although he is cleared to take a reduced dose of acetaminophen as needed for everyday pain, he avoids all over-the-counter pain relievers—especially aspirin—as well as alcohol, to protect his recovering liver. "If I get a headache, I will grin and bear it," he states resolutely.

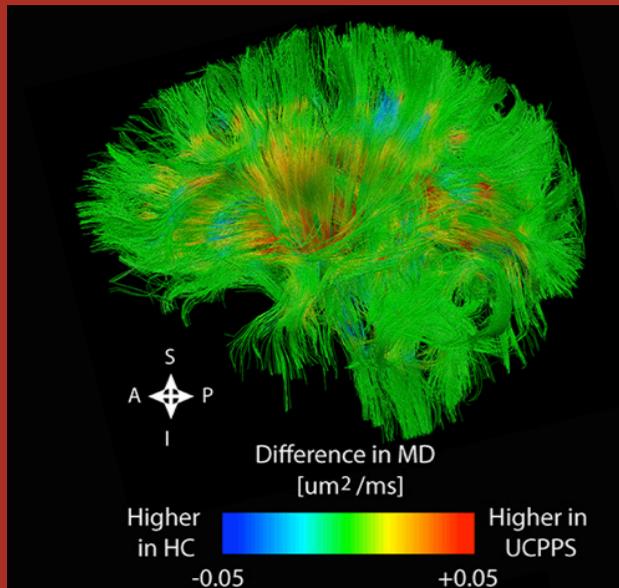
"It was a scary experience," Scott says, summing up his ordeal with acute liver failure. "But I definitely had faith in the doctors," he adds appreciatively.

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

Overall, his experience with participating in the STOP-ALF clinical trial was a positive one that Scott would recommend to others who might find themselves in similar circumstances. "If it helps

other people, I'm more than happy to participate and do follow-up," he says, though he urges anyone considering

enrolling in a clinical trial to be fully informed, including asking the medical staff any questions they might have. "It was a scary experience," he says, summing up his ordeal with acute liver failure. "But I definitely had faith in the doctors," he says appreciatively. And, he adds, "every time I've gone back to do the follow-up, I'll check in on the ward and see some of the nurses there just to thank them."



Studies have shown that chronic pain conditions are often accompanied by alterations in brain structure and function. Research described in this chapter suggests that there may be brain changes unique to people with urologic chronic pelvic pain syndromes (UCPPS). Magnetic resonance imaging (MRI) is a noninvasive approach being used to try to identify unique sets of changes, or “brain signatures, for different pain conditions, as these could lead to improved understanding, diagnosis, and therapy. This image depicts a model of the brain constructed by combining highly detailed MRI scans obtained

from people with UCPPS and from healthy people. Areas in green showed no structural differences, whereas the areas in blue, yellow, and red scattered throughout the brain differed between people with UCPPS and healthy individuals. This is one of several images that, with accompanying analyses, enabled researchers to identify a number of microstructural changes in the brains of people with UCPPS, particularly in areas related to perceiving and responding to pain, some of which may be unique to people with UCPPS as compared to people with different chronic pain syndromes.

*Image courtesy of Dr. Benjamin Ellingson, University of California, Los Angeles. Originally published in Woodworth D, et al. Unique microstructural changes in the brain associated with urological chronic pelvic pain syndrome (UCPPS) revealed by diffusion tensor MRI, super-resolution track density imaging, and statistical parameter mapping: a MAPP Network neuroimaging study. *PLoS ONE* 10(10): e0140250, 2015. Reprinted under a Creative Commons Public Domain Dedication (<https://creativecommons.org/publicdomain/zero/1.0/>).*

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

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated. CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2014, over 678,000 patients received treatment for ESRD: over 467,000 received either hemodialysis or peritoneal dialysis, and over 196,000 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 some of the 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 and developing treatment strategies. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, 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

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

² U.S. Renal Data System, *USRDS 2016 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, Maryland, 2016.

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 Institute issued a research solicitation titled “Kidney Precision Medicine Project” that aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury (AKI) or CKD for the purpose of creating a kidney tissue atlas, defining disease subgroups, and identifying critical cells, pathways, and targets for novel therapies. In addition, NIDDK issued a research solicitation titled “Pilot Clinical Trials in Pediatric Chronic Kidney Disease” to form a multi-center collaboration to perform pilot trials to optimize study designs for larger trials for new pediatric CKD treatment.

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

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

and mitigate ureteral stent-related pain and symptoms; and c) provide data and collect biological samples from the studies to create a resource for future researchers. Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPSP) in men, are also important research topics of the NIDDK’s urology program.

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

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

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

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 12-7865, 2012.

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 established the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health, established the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal or healthy bladder function and to identify behavioral and other risk factors for conditions associated with lower urinary tract symptoms in women.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and of chronic diseases. To promote high-impact basic or pre-clinical research, the Institute supports the Stimulating Hematology Investigation: New Endeavors (SHINE) program with current topic areas: hematopoietic stem cell determinants, non-coding RNA, macrophages, and aging.

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.

KIDNEY FORMATION AND FUNCTION IN HEALTH AND DISEASE

Mining the Genome for Insights into Kidney

Function and Development: An international group of researchers, examining data from more than 230,000 people, have discovered 24 new areas of

the genome (the entire set of genetic information) that are associated with kidney function or development and have confirmed 29 other genomic areas that were previously identified. Estimates suggest that more than 20 million adults in the United States have chronic kidney disease (CKD) of varying levels of seriousness. To identify genes associated with CKD, a team of scientists scanned the genomes of more than 130,000 people of European ancestry for genetic variants associated with estimated glomerular filtration rate (eGFR), a measurement of how well their kidneys are filtering wastes and extra fluid from the blood. The researchers identified 24 new regions of the genome that were associated with kidney function and confirmed the findings by further analysis in more than 42,000 additional people of European ancestry. Their analysis also confirmed 29 genomic regions already known to be associated with eGFR. Many of these genomic regions were associated with eGFR in people with type 2 diabetes or high blood pressure—populations at particularly high risk for CKD. The scientists also examined the genomes of over 16,000 people of African ancestry and more than 42,000 Asians. Several of the newly identified regions were associated with kidney function in other ethnic groups, suggesting that these results likely extend beyond people of European ancestry. Computational analyses determined that genes found to be in these genomic regions were mostly turned on in cells within the kidney or urinary tract, and were involved in processes related to kidney development and function. The specific genomic variations associated with eGFR often resided in portions of the genes that determine when, where, and to what extent the genes are turned on. Additional studies will be necessary to understand the precise role of each genomic region in kidney development and physiology, but these findings have generated numerous potential targets for therapeutic strategies to improve kidney health, including in people with type 2 diabetes or high blood pressure.

Pattaro C, Teumer A, Gorski M, ...Fox CS. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. Nat Commun 7: 10023, 2016.

A Cause of Hardening and Narrowing of Arteries in

Kidney Disease Identified: Scientists have found in mice that a certain type of stem cell contributes to artery "hardening" that can lead to cardiovascular events like heart attacks and strokes. Arterial

calcification, or “hardening” of the arteries due to the accumulation of minerals in the artery walls, is often a precursor to cardiovascular disease, especially when accompanied by atherosclerosis, or narrowing of the arteries due to formation of fatty “plaques” on the inner walls. These conditions are common among those who have chronic kidney disease (CKD) and can lead to dangerous blood clots and/or heart failure. Mesenchymal stem cells (MSCs) in the arteries have been thought to contribute to artery disease and repair, but research on these cells has been hindered by a lack of tools to follow their fate as they differentiate, or mature into more specialized cells. To study MSCs’ role in artery health, researchers identified a protein marker, called Gli, which could be used to identify arterial MSCs experimentally. These Gli⁺ stem cells normally reside in the outer walls of the arteries, but in a mouse model of arterial injury they differentiated and migrated to wound sites to take part in wound repair. The scientists then used a mouse model to investigate what role these Gli⁺ cells might play in disease. In a mouse model of CKD that includes artery hardening from calcification and fatty plaques, the Gli⁺ cells migrated into the arteries to cluster around arterial plaques and differentiated into osteoblasts, or bone-forming cells. When researchers genetically engineered mice to have significantly reduced numbers of Gli⁺ cells, these mice had significantly less artery calcification than normal mice, suggesting that the Gli⁺ cells were responsible for artery hardening. These results may also have important implications for people. Researchers found Gli⁺ cells in the arterial plaques of men with CKD, while the Gli⁺ cells in the arteries of men without CKD stayed in the outer arterial walls. Overall, these findings gave important new insights into stem cell biology and may offer new targets for treatment or prevention of arterial disease.

Kramann R, Goettsch C, Wongboonsin J, ...Humphreys BD. Adventitial MSC-like cells are progenitors of vascular smooth muscle cells and drive vascular calcification in chronic kidney disease. Cell Stem Cell 19: 628-642, 2016.

Decline in Nephron Numbers in the Kidney Even in Healthy Aging: New research shows that from young adulthood to old age, healthy adults lose about half of their nephrons—the basic functional unit of the kidney. Nephrons consist of various cells and structures that work together to filter waste products and excess fluid from the blood; the glomerulus is the fundamental filtering apparatus in the nephron. Previous research

has shown that the number of nephrons correlates with the functional capacity of the kidney, and that nephron loss occurs with aging. However, most studies estimating nephron number have been done using kidney samples obtained at autopsy rather than from living people, as the only way to obtain kidney tissue from living people is through an invasive biopsy. Researchers took advantage of the fact that people donating one of their kidneys to another person—*i.e.*, living kidney donors—undergo tests before and during the transplant procedure, including a kidney biopsy. They used data from these tests to estimate nephron numbers in 1,638 healthy male and female living kidney donors, ages 18 to 75.

The researchers found that donors who were 18 to 29 years old had about 990,000 nephrons. In contrast, 70- to 75-year-olds had about 520,000 nephrons, or roughly half the number as in the younger group. Overall, nephron number was found to decrease by about 7 percent every decade from young adulthood to old age. Men had more nephrons than women, but there was no difference in the age-related decline in nephron number between men and women. The researchers also observed a decrease in glomerular filtration rate (GFR)—a measure of kidney function—with age that correlated with the age-related decline in nephron number. In addition to older age and lower GFR, other clinical characteristics associated with lower nephron number included shorter height, family history of end-stage renal disease, and higher level of uric acid in the blood. The researchers also discovered that using other methods to estimate nephron number, such as by measuring the volume of the outer rim of the kidney (the cortex) by computed tomography scan, grossly underestimated nephron loss.

These findings show that there is substantial nephron loss as a part of aging in healthy adults. The researchers note that current clinical guidelines use a single GFR threshold for diagnosing kidney disease. However, because of the observed nephron loss with healthy aging and the correlating decline in GFR, using a single cut-off value may over-diagnose kidney disease in older adults (who have a lower GFR compared to younger adults) and under-diagnose kidney disease in younger adults. The researchers also note, however, that the population included in this study was predominantly White and had only 11 participants aged 70 to 75 years, so additional research is needed to assess generalizability of the findings. Further

research, including developing improved imaging techniques that enable nephron number to be estimated in living people without the need for a biopsy, could help shed light about nephron number in other populations and how to consider nephron loss in healthy aging when diagnosing kidney disease.

Denic A, Lieske JC, Chakkera HA, ...Rule AD. The substantial loss of nephrons in healthy human kidneys with aging. *J Am Soc Nephrol* 2016 Jul 8. pii: ASN.2016020154. [Epub ahead of print]

Understanding the Role of an Important Family of Genes in Human Kidney Development: New research has elucidated important differences between mouse and human kidney development in the role of a family of genes—findings that could explain why people have more nephrons than do mice, and could inform new therapeutic strategies for the prevention and treatment of kidney disease.

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. The mechanisms driving the number of nephrons are particularly important to determine because low nephron number has been found to be associated with increased risk for high blood pressure and reduced kidney function in people. Humans have a vastly higher number of nephrons per kidney than do mice (1 million versus 13,000, on average). Key distinctions in kidney development could account for this difference, including the fact that nephron formation takes significantly longer to complete in humans than it does in mice.

To explore the differences in kidney development, researchers investigated two members of the “Six” gene family, *Six1* and *Six2*, which encode regulatory proteins known to be essential for proper kidney development in the mouse, but not well explored in human developmental systems. In mouse and human kidney development, the *Six2* and *SIX2* genes, respectively, are turned on in a pool of progenitor cells that give rise to nephrons throughout the period of nephron generation. Previous studies have shown that the mouse *Six1* gene is turned on only transiently in early stages of kidney development. By contrast, the researchers found that the human *SIX1* gene is similarly turned on early, but also continues throughout the period of extensive nephron formation. They also

determined that, specifically in humans, the activation of the *SIX1* gene is regulated, in part, by the protein that is encoded by the *SIX2* gene. These findings reveal a divergence between mice and people in the molecular regulators controlling the fates of nephron progenitor cells. The scientists hypothesize that the expanded activation of the *SIX1* gene in humans could play a role in maintaining the pool of progenitor cells, which could then establish the higher number of nephrons observed in humans compared with mice. Additional research could address that possibility.

Often, translating important discoveries from mouse models to applications in humans can be challenging due to differences between species. Armed with this new knowledge on human kidney development, scientists could develop novel strategies to increase nephron number in people, and test whether this would improve kidney function.

O'Brien LL, Guo Q, Lee Y, ...McMahon AP. Differential regulation of mouse and human nephron progenitors by the Six family of transcriptional regulators. *Development* 143: 595-608, 2016.

Technological Improvements Help Transplanted Kidney Tissue Connect with a Host's Circulatory System: Researchers have identified new experimental conditions that improve the viability of transplanted kidney tissues and their ability to interact with the host blood supply in rodent models. For people with kidney failure, the two main treatment options are dialysis or a kidney transplant. However, there is currently a significant shortage of donated kidneys that are available for transplantation. Therefore, research towards improving methods for engineering functional kidney tissues remains an urgent public health undertaking. Previous attempts in rodent models to transplant engineered kidney tissues have met with limited success, partly because the hosts' vascular systems could not seamlessly connect with the transplanted tissue.

In a recent study, scientists addressed this problem by isolating rat glomeruli (balls of capillaries through which the blood is filtered in the kidney) that were suspended in an engineered matrix and transplanted into host female mice. They compared the viability of transplanted glomeruli with and without the addition of human endothelial cells (ECs) that were genetically modified to produce the cell survival protein Bcl-2. ECs

form the inner lining of blood vessels. These genetically modified cells, which spontaneously form small blood vessels in a matrix suspension, were termed Bcl-2-ECs. After 2 weeks post-transplantation, overall survival of rat glomerular cells in the presence of Bcl-2-ECs was about 10 percent, whereas almost no glomeruli survived without Bcl-2-ECs. Using injected dyes, the researchers then found that the glomerular capillaries were capable of connecting to the newly formed blood vessels derived from the Bcl-2-ECs. Furthermore, 15 days after transplantation, all viable glomeruli suspended in the presence of Bcl-2-ECs were acquiring blood from the host mouse's circulatory system. However, a closer look at the transplanted glomeruli by electron microscopy revealed a few structural problems in some of the cells. Together, these data uncover an important technological advance in generating viable kidney tissue that can connect with a recipient's blood vessels after transplantation. Although this new knowledge represents an important step forward, additional research will be needed to improve the efficiency, integrity, and function of transplanted engineered kidney tissues.

Chang WG, Fornoni A, Tietjen G,...Pober JS. Tissue-engineered microvasculature to reperfuse isolated renal glomeruli. Tissue Eng Part A 21: 2673-2679, 2015.

KIDNEY STONE TREATMENT

Moving Stones with Sound—New Ultrasound Technology Repositions Kidney Stones in People:

Researchers have developed new ultrasonic propulsion technology that can reposition kidney stones and facilitate stone fragment passage in people. Kidney stones are one of the most common disorders of the urinary tract. Smaller stones may pass with little or no pain, while larger stones may get stuck along the lower urinary tract and block the flow of urine, causing severe pain and/or bleeding. Current treatments for kidney stones, such as lithotripsy, may leave behind residual stone fragments. Most fragments will pass on their own, but others may grow larger, cause pain, and lead to the need for additional treatment.

Toward the goals of finding safe ways to reposition kidney stones and encouraging the passage of stone fragments, scientists developed ultrasonic propulsion technology. The technology uses a handheld device

to generate a real-time ultrasound image to visualize the kidney stone, and directs controlled, short bursts of ultrasound waves toward the stone to try to make it move. In the first human clinical trial testing this technology, scientists found that it could reposition kidney stones in 14 of 15 men and women studied, and cause some degree of movement of both large and small stones. In fact, one person experienced pain relief after a large, obstructing stone was moved. These findings suggest that the procedure could successfully reposition kidney stones in some people. The scientists then examined six study participants who had residual stone fragments after previously undergoing a lithotripsy procedure to treat their kidney stones. Four of them passed more than 30 fragments within days after undergoing the ultrasonic propulsion procedure, demonstrating that the technology could facilitate the passage of stone fragments. An unexpected finding was that the technology may also be useful for diagnosis—in four people, what was thought to be one large stone was actually found to be a cluster of small, passable stones after they were moved. Stone size is an important factor that doctors consider when making treatment decisions, so having this diagnostic information could aid them in making those decisions. Importantly, the technology was found to be safe and did not cause pain. It is also noninvasive and could be performed in a clinic setting while people are awake without the need for sedation. Ultrasound propulsion technology is still being refined and tested in people, but with further research, it may eventually be possible to use this new technology after procedures that leave residual stone fragments to facilitate their passage and potentially reduce the need for future intervention. The technology may also be useful for moving large, obstructing stones; repositioning stones before surgery; and serving as a diagnostic tool.

Harper JD, Cunitz BW, Dunmire B,...Bailey MR. First in human clinical trial of ultrasonic propulsion of kidney stones. J Urol 195: 956-964, 2016.

New Approach May Reduce Risk of Kidney Stone Formation: A molecular tool called RNA interference (RNAi) has been shown to be effective in reducing oxalate production in animal models of primary hyperoxaluria (PH), a rare, inherited condition characterized by recurrent kidney and bladder stones. PH type 1 often results in end-stage renal disease (ESRD), a life-threatening condition in which the kidneys

can no longer filter fluids and eliminate waste products from the body effectively. People with PH type 1 have excess oxalate in their bodies because they lack a liver protein that would normally prevent oxalate overproduction. Most kidney stones consist mainly of crystallized calcium oxalate and small amounts of other compounds. Both calcium and oxalate are components of a normal diet, and a high level of oxalate in the urine correlates with increased risk of stone formation. However, decreasing dietary intake of oxalate has not been demonstrated to be effective in preventing kidney stone formation. Although surgical interventions, such as combined liver-kidney transplantation, represent an option for a small number of patients, there are no FDA-approved medically based therapeutic approaches for the treatment of PH type 1. Moreover, dialysis does not adequately remove oxalate from the body.

A recent report describes how scientists explored the use of RNAi to block production of oxalate. RNAi is a widely used technique that allows researchers to target and “silence” a gene of interest so that the protein it encodes is not made. Recently, the technique has begun to be harnessed toward possible therapeutic applications. In this study, researchers took advantage of what is known about the multi-step pathway to oxalate production, in which a molecule called glycolate is converted to another molecule, glyoxylate, which can be converted to oxalate. Glycolate is converted to glyoxylate in liver cells by an enzyme called glycolate oxidase, or GO. Because people with PH type 1 do not metabolize glyoxylate normally—which causes it to build up and be converted to oxalate—the RNAi compound “ALN-GO1” was selected to silence the gene that encodes GO. The team hypothesized that, in the absence of GO, glycolate would accumulate, but be readily and safely eliminated from the body in the urine, while the remainder of the pathway would effectively shut down, preventing the excess oxalate production that causes harm in PH type 1.

Administration of ALN-GO1 significantly silenced the gene encoding GO in normal male mice, rats, and monkeys. In normal male monkeys and in a mouse model of PH type 1, ALN-GO1 increased glycolate levels in urine. Notably, and as predicted, ALN-GO1 significantly reduced urinary oxalate levels in both a mouse model of PH type 1 and a rat model that normally exhibits high levels of oxalate in the urine. These preclinical findings support further investigation

into whether ALN-GO1 may have the potential to benefit patients with PH type 1.

Liebow A, Li X, Racie T,...Knight J. An investigational RNAi therapeutic targeting glycolate oxidase reduces oxalate production in models of primary hyperoxaluria. *J Am Soc Nephrol* 2016 Jul 18. pii: ASN.2016030338. [Epub ahead of print]

KIDNEY TRANSPLANTATION

Promising Result Reported from Multi-center

Kidney Transplantation Study: Researchers have reported a survival benefit for people who received kidney transplants from HLA-incompatible live donors compared with either those remaining on the kidney transplant waiting list or those who received kidney transplants from immune system-compatible deceased donors. Human leukocyte antigen (HLA) is a protein on the surfaces of human cells that identifies the cells as “self” or “foreign,” and performs essential roles in immune responses. There are multiple forms of HLAs, which vary among individuals and are analyzed in laboratory tests to determine whether one person’s organs and tissues are compatible with another person’s, and could be used in a transplant. The more closely the HLAs match between a donor and recipient, the less likely a transplant will be rejected by the recipient’s immune system. To overcome HLA-incompatible transplants, organ transplant recipients undergo “desensitization” protocols to remove antibodies in the blood that can harm the donated organ. Previous research from a single center indicated a survival benefit with kidney transplants from HLA-incompatible live donors as compared with those waiting for a compatible organ.

To assess whether the survival benefit seen in the single-center study is generalizable on a national scale, a 22-center study was designed and conducted. The researchers assessed the survival of people who received kidney transplants from HLA-incompatible live donors, at multiple time points up to 8 years after transplantation. They compared these outcomes with the survival of two control groups—those who remained on the waiting list or received a transplant from a deceased donor, and those who remained on the waiting list but did not receive a transplant. The multicenter study reported that a kidney transplant from an HLA-incompatible live donor was associated

with a significant survival benefit compared to the two control groups. As a compatible live kidney donor is rarely available, these results suggest that patients now could consider the option to undergo incompatible transplantation.

Orandi BJ, Luo X, Massie AB, ...Segev DL. Survival benefit with kidney transplants from HLA-incompatible live donors. *N Engl J Med* 374: 940-950, 2016.

INSIGHTS INTO UROLOGICAL PAIN SYNDROMES

Evaluating Nervous System Involvement in Women

with Chronic Pelvic Pain: Researchers have gained some new insights into nervous system contributions to chronic pelvic pain conditions in women. Nerves that are part of the autonomic nervous system (ANS) regulate bodily activities that generally run without conscious thought, such as heart rate, blood pressure, digestion, and bladder function. If ANS nerves malfunction or become damaged, however, a person can experience symptoms such as dizziness, increased or decreased sweating, and problems with urination. Researchers had previously detected possible malfunction, although not damage, in the ANS of women with interstitial cystitis/bladder pain syndrome (IC/BPS), a chronic urologic pelvic pain syndrome whose symptoms include urinary frequency, urgency, and pain with bladder filling. However, those initial results were complicated by the fact that many of these women also had another, distinct pelvic pain problem unrelated to bladder function, called myofascial pelvic pain (MPP).

In the present study, the same research team sought to distinguish ANS problems in women with IC/BPS versus other pelvic pain. Study volunteers included women with IC/BPS, women with MPP, women with both, and healthy women without either condition. These different groups of women all participated in a series of tests designed to detect the presence and extent of ANS abnormalities. For example, one test involved lying flat for several minutes on a tilt table that was subsequently raised partway upright, enabling the researchers to measure how effectively participants' bodies adjusted to the tilt-associated drop in blood pressure. Other tests included breathing tests to measure heart rate and blood pressure, and a sweat test. The results of the tests suggest that women with MPP experience one type of ANS damage, autonomic neuropathy, more frequently than women with IC/BPS only.

Intriguingly, many of the women with any chronic pelvic pain—but none of the healthy women—reported symptoms such as dizziness when moved upright in the tilt-table test, but without any measurable changes in vital signs. This result suggests that the women's symptoms were related to a heightened awareness of or sensitivity to environmental stimuli that can occur in pain conditions, rather than to ANS abnormalities. Finally, the researchers noted an increase in baseline heart rate among all the women with pelvic pain conditions compared to healthy women. Combining this result with observations from their earlier study, they suggest that women with chronic pelvic pain, particularly women with IC/BPS, may have systemic neural changes rather than nerve problems restricted to, for example, the bladder. The indicator of this systemic change, called vagal tone withdrawal, can potentially be addressed therapeutically.

Clarifying underlying causes of symptoms in chronic pain conditions is helpful for clinical treatment; thus, these new insights into the variable involvement of the ANS in chronic pelvic pain conditions in women could be helpful in future therapeutic strategies.

Chelimsky G, Simpson P, McCabe N, Zhang L, and Chelimsky T. Autonomic testing in women with chronic pelvic pain. *J Urol* 196: 429-434, 2016.

Unique Microstructural Changes in Brains of People with Urologic Pain Syndromes Revealed:

Scientists using advanced imaging technology have identified a number of minute and specific brain changes associated with urologic chronic pelvic pain syndromes in people. Research has shown that people with chronic pain conditions, including people with the urologic chronic pelvic pain syndromes (UCPPS) interstitial cystitis/bladder pain syndrome or chronic prostatitis/chronic pelvic pain syndrome, exhibit changes in various brain regions, many related to pain perception. However, there is also reason to believe that unique sets of changes, or "brain signatures," can be identified for specific chronic pain conditions, potentially leading the way to improved understanding, diagnosis, and therapy for these conditions.

Building on prior studies, researchers in the NIDDK's Multidisciplinary Approach to the Study of Chronic Pelvic Pain Research Network used noninvasive magnetic resonance imaging (MRI) technology in two different ways to capture as much information as possible that might reveal such brain signatures in men and women with UCPPS.

Using these approaches, they compared brain images between people with UCPPS, healthy people, and people with a different visceral pain syndrome, irritable bowel syndrome (IBS). They found several microstructural differences throughout the brain between people with UCPPS and healthy people, including in areas involved in perceiving and responding to pain. By comparing the images from people with UCPPS and people with IBS, they could see that the changes were not identical between these two pain syndromes. Combining these analyses, the team identified at least three brain regions with changes that differed between people with UCPPS and the two other groups. They also looked to see whether any of the observed brain changes correlated with UCPPS symptom severity and/or duration, and found a few that did. This finding also suggests that the observed brain changes are a response to, rather than precede, chronic pain. Finally, because there are known sex-based differences in pain and pain syndromes, they also examined whether, with their approaches, they could see differences in the brain microstructure between women and men in any of the three groups. Interestingly, while a variety of male/female differences were observed within each group, the fewest differences were seen among those with UCPPS—raising the possibility that, if therapies emerge based upon “brain signatures,” men and women with UCPPS may benefit from similar treatments.

This exploratory research suggests that microstructural brain alterations specific to UCPPS exist. Future research may now focus on such questions as whether other changes can be detected, whether they and/or the changes observed in this study vary over time or in response to symptom changes or treatments, and whether one or more of these changes might emerge as a useful biomarker or “signature” for UCPPS versus other pain syndromes.

Woodworth D, Mayer E, Leu K,... Ellingson BM; for the MAPP Research Network. Unique microstructural changes in the brain associated with urological chronic pelvic pain syndrome (UCPPS) revealed by diffusion tensor MRI, Super-resolution track density imaging, and statistical parameter mapping: a MAPP Network neuroimaging study. *PLoS One* 10: e0140250, 2015.

UNDERSTANDING HEMATOLOGICAL DISEASES

Reving Up Human Red Blood Cell Production: A recent study demonstrates that “turning off” a single gene significantly increased production of human red blood

cells (RBCs) in the laboratory. The mechanisms controlling the transition from embryonic stem cells (ESCs) or early stage (progenitor) blood cells to mature RBCs are not well understood, but such knowledge could provide critical insight into how to produce blood cells in the laboratory.

While characterizing samples obtained from 4,678 volunteers, researchers discovered 11 rare mutations in the *SH2B3* gene associated with higher hemoglobin and hematocrit levels. Hemoglobin carries oxygen in RBCs from the lungs to the rest of the body, and the hematocrit measures the percentage of the blood that consists of RBCs. Thus, people who have a rare *SH2B3* mutation have higher levels of RBCs in their blood.

To confirm that the *SH2B3* genetic mutation was responsible for the increased RBC production, two different genetic approaches were used to essentially block the function of the gene in human ESCs and progenitor cells, which can mature to become various types of blood cells. The maturation of human ESCs and progenitor cells into RBCs is accomplished in the laboratory by the addition of a cocktail containing various factors identified from previous research. In the first approach, they used a technique called RNA interference to prevent cells from making the protein encoded by the *SH2B3* gene by disrupting a key intermediate in the protein-making process, a copy of the gene made of RNA. The scientists found that this approach successfully increased RBC production by at least three-fold. The increased RBC production was due to both increased maturation and increased production, and the newly produced RBCs appeared to have similar size and shape to natural RBCs. In the second approach, the *SH2B3* gene was inactivated in a line of human ESCs by the CRISPR/Cas9 system, which enables the gene’s DNA to be edited with unprecedented precision. (The NIH supports research using human ESCs within the *NIH Guidelines for Human Stem Cell Research*.) The results of this set of experiments showed that ESCs lacking a functional *SH2B3* gene produced approximately three-fold more RBCs than human ESCs having the intact gene. Although the RNA interference method would be difficult to scale-up to produce sufficient quantity of RBCs for clinical use, the CRISPR/Cas9 system could permanently shut-off the *SH2B3* gene in a renewable cell line, which could help enable larger-scale production of blood cells. Thus, the results of two different genetic approaches identify the *SH2B3* gene as having a mechanistic role in the ability of stem and progenitor blood cells to develop into RBCs in the laboratory.

This newly acquired knowledge may contribute to future efforts to improve RBC production for medical applications such as replacement therapy during acute blood loss as a result of trauma or surgical procedures. This research may also lay the foundation to produce cells of rare blood types for people who need very specific types of blood not available via donated blood resources.

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The Double-edged Sword of a Pro-inflammatory Protein:

New research delineates acute versus chronic effects of interleukin-1 (IL-1) exposure on blood stem cell fate. Inflammation is one of the immune system's responses to insults such as a bacterial infection or a splinter piercing a finger. Many different immune cells can take part during the process of inflammation. The immune cells release substances, also called inflammatory mediators, that direct blood stem (precursor) cells toward one of two pathways—production of more stem cells (self-renewal) or maturation into specialized cell types. One such mediator is IL-1, which plays a central role in fighting infections. IL-1 was previously shown to be an “emergency” signal to increase numbers of certain types of blood cells when needed. However, the consequences of acute (short duration) versus chronic (long-lasting) exposure of blood stem cells to IL-1 is largely unknown. Because chronic inflammation is a feature of a number of diseases and conditions, further understanding of inflammatory mediators, like IL-1, may lead to new ideas for treatment strategies.

To learn more about the way IL-1 exerts its effect, researchers designed studies using both isolated mouse blood stem cells and normal male and female mice. Under conditions of acute exposure, IL-1 significantly increased the rate of blood stem cell division into new daughter cells. IL-1 directed the new daughter cells to mature into blood cells called macrophages and granulocytes—members of the so-called myeloid family of blood cells—which have the ability to ingest and degrade invading bacteria. Consistent with findings using isolated cells, when the researchers injected mice once (acute exposure) with IL-1, they found a rapid increase in the percentage of myeloid cells in circulating blood, while levels of lymphoid cells—which include T cells and B cells of the immune system—and red blood cells declined. To assess IL-1 action on blood cell production on chronic, as compared to acute exposure, the researchers injected mice once or once daily for 20

days (chronic) and determined the levels of myeloid and lymphoid cells in bone marrow. The researchers reported a loss of early stage lymphoid cells following a single IL-1 injection. Following 20 days of IL-1 injections, there was a significant change in the composition of bone marrow cells—a significant increase in myeloid cells and a significant decrease in lymphoid cells. Thus, chronic IL-1 exposure alters the bone marrow's ability to maintain a balanced blood cell population. The researchers also found that IL-1 impairs blood stem cell self-renewal. In humans, this could negatively affect the outcomes of anti-cancer procedures such as autologous stem cell transplants, which require the regrowth of a patient's immune system from his or her own functional stem cells. Notably, the researchers found that upon IL-1 withdrawal, the bone marrow will “reset” to the numbers of myeloid and lymphoid cells and the capacity for stem cell self-renewal observed prior to IL-1 treatment.

This study, using isolated mouse blood stem cells and mice, identified IL-1 as a critical regulator of blood stem cell fate. Future research will be necessary to determine whether IL-1 underlies blood stem cell fate and changes in numbers and function of in blood cells in the context of chronic inflammation in humans.

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Ramping Up Fetal Hemoglobin Production—Implications for Red Blood Cell Diseases:

Research teams have provided new information regarding how DNA binding proteins help “turn off” production of fetal hemoglobin (HbF), which may lead to new ways to “turn on” HbF production to treat certain red blood cell diseases. People with one such disease, sickle cell disease, suffer from chronic anemia and episodes of bone, joint, and muscle pain, as well as other complications, because their red blood cells form rigid, “sickle” shapes in small blood vessels, leading to shortened red blood cell survival and impaired blood flow and oxygen delivery to tissues. This disease results from genetic mutations that affect the form of hemoglobin often called “adult” hemoglobin, even though its production begins soon after birth. Individuals with another genetic disorder of hemoglobin, β -thalassemia, also suffer from chronic anemia caused, in their case, by impaired adult hemoglobin production, which results in reduced numbers and viability of red blood cells.

Although HbF is mostly undetectable in adults and children (after about 6 months of age) in the general population, increased levels safely persist to varying degrees in some people. Researchers have observed that people with sickle cell disease who retain higher levels of HbF have less severe disease. Thus, one potential treatment approach is to reactivate HbF production, with the hope that, at sufficient levels, it could compensate for both the defective function of adult hemoglobin in sickle cell disease and the impaired synthesis of adult hemoglobin in β -thalassemia.

Previous research has shown that the DNA binding protein BCL11A prevents the production of HbF and that when BCL11A is absent, HbF levels increase. Building on these research findings, the current study explored the enhancer region of the *BCL11A* gene—a DNA element that helps “turn on” the gene—to see whether changes in the enhancer would “turn off” BCL11A’s production. To test systematically the importance of different parts of the enhancer, the researchers used a gene-editing tool to remove tiny sections of DNA along the length of the enhancer in stem cells or progenitor cells. These early stage cells were allowed to mature into red blood cells and tested for their ability to produce HbF. The deletion of a discrete region termed “h+58” within the human *BCL11A* enhancer resulted in decreased BCL11A production and increased HbF production. These results provide the foundation for the potential use of therapeutic genome editing of the *BCL11A* enhancer to raise the levels of HbF in people with hemoglobin-related diseases (hemoglobinopathies).

Another approach to “turn off” BCL11A production involves the use of small hairpin RNAs (shRNAs). The genetic information stored within DNA is encoded into messenger RNAs (mRNAs), which in turn are translated into proteins. Translation of mRNA into proteins can be “silenced” by synthetically produced, hairpin-shaped shRNA molecules which can be designed to cause the degradation of specific mRNAs. Targeting BCL11A mRNA in this way, for example, would prevent BCL11A protein from being made. In this recent study, investigators used an experimental system to deliver DNA encoding shRNAs targeting BCL11A to both human and mouse blood stem cells, where the shRNA was then produced. Initially, they designed the system for shRNA to be produced at high levels in many types of cells. The researchers’ findings showed that though this system reduced BCL11A protein levels, it also caused unwanted side-effects in both human and mouse cells: decreased numbers of blood cells—B cells, monocytes, and granulocytes—once the shRNA-treated

cells were transplanted into living animals. The decreased numbers of these various blood cells were attributed to loss of BCL11A function in these cells and would be a major hurdle for future therapeutic use of this system. Researchers hypothesized that they could overcome the negative impact of BCL11A loss in B cells, monocytes, and granulocytes by targeting the BCL11A-specific shRNA production only to red blood cells. To test this, they designed a system that would only produce shRNA in progenitor and mature red blood cells and thus would cause red blood cell-specific loss of BCL11A protein. Using this new “lineage-specific” approach, the investigators reported normal levels of transplanted B cells, monocytes, and granulocytes while maintaining the desired effect of reducing BCL11A protein and increasing HbF production in red blood cells. Furthermore, when they used this lineage-specific system to reduce BCL11A in mouse blood stem cells, and then transplanted those cells into a mouse model of sickle cell disease, they observed a substantial reduction in disease markers.

In addition to BCL11A, the DNA binding protein LRF has recently been identified as having the ability to modulate HbF production. LRF was initially discovered in this study for its ability to prevent production of HbF; when the gene that encodes LRF was genetically inactivated in red blood cells of adult mice, HbF levels increased. The investigators further showed that LRF binds to the HbF gene in a way that prevents production of HbF. Although both BCL11A and LRF bind to the *HbF* gene, they apparently do so in different ways, demonstrating that these proteins share common features yet have distinct mechanistic actions.

Together, these studies contribute new knowledge about BCL11A and LRF control of HbF production and may enable the development of safe and effective gene therapies to reactivate HbF production in patients with hemoglobinopathies.

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The Chronic Renal Insufficiency Cohort Study



Chronic kidney disease (CKD) is a major public health problem in the United States. The impact of CKD is substantial and includes increased risk of death, diminished quality of life, numerous co-associated diseases and conditions, such as cardiovascular disease (CVD, which includes heart disease and stroke), and significantly increased risk of progression to kidney failure (end-stage renal disease). As symptoms are few or non-existent, most people are unaware they have CKD until most kidney function has been lost. Understanding the risk factors for progression of CKD and associated CVD is necessary to design clinical trials, and identify candidate therapies to be tested in clinical trials to reduce the impact of CKD. This requires detailed evaluation and long-term follow-up of individuals with significantly diminished kidney function.

To identify the risk factors for loss of kidney function and the link between kidney and heart diseases, the NIDDK established the Chronic Renal Insufficiency Cohort (CRIC) observational study in 2001. This epidemiologic study seeks to study the distribution and determinants of health-related events in the CKD population and apply this acquired knowledge to improving health. Between 2003 and 2008, CRIC recruited nearly 4,000 men and women with CKD, about one-half of whom are African American and approximately one-half of whom reported they had diabetes. This cohort included 327 Hispanic Americans with CKD recruited through an ancillary project designed to augment the CRIC Study's ability to assess this large and growing population. From 2013-2015, CRIC recruited a second cohort of nearly 1,600 men and women. This second cohort is older and on average has a higher level of kidney function than the first cohort, and enables the study of frailty, other features of accelerated

aging, and the identification of risk factors for loss of kidney function earlier in the course of CKD. All study participants have annual in-clinic visits consisting of standard blood, urine, and other tests measuring kidney, heart, and vascular health as well as an interim telephone contact between clinic visits. Because very few of the participants have dropped out, the CRIC study has generated a very complete and robust database that is being used by the research community.

To leverage the NIDDK's investment in CRIC, over 80 ancillary studies are either ongoing or have been completed. Most of these are investigator-initiated research projects to further analyze data and participants from the CRIC study. This effort has not only expanded the scope of the science conducted by CRIC investigators, but also has increased the number of investigators and institutions affiliated with this long-term epidemiological study. To further educate and engage the research community, the CRIC study group conducts annual data workshops to facilitate the use/analysis of the CRIC data available through collaborative ancillary studies and through the NIDDK Central Repository.

Over 130 research papers have been published to date describing CRIC findings. Highlights include the following notable research advances:

Key Link Discovered Between Kidney Disease and Heart Disease: CRIC investigators have reported that high levels of a hormone called FGF-23, which regulates phosphate metabolism, are associated with an increased risk of CVD in patients with CKD. Elevated FGF-23 levels were shown to be associated

with increased risk of structural heart disease (abnormal elevations in the size of the heart's left ventricle) and heart failure (insufficient pumping of the heart that leads to retention of body fluids and congestion of the lungs). Experiments conducted in animal models supported the findings found in CRIC Study participants. For example, mice developed enlarged left ventricles following injection of FGF-23. This and other experiments suggest that FGF-23 may play a direct causal role in the heart disease seen so commonly in the setting of CKD.

Genetic Variation and Progression of Chronic Kidney Disease: CRIC investigators, in collaboration with other researchers, reported that *APOL1* gene variants significantly contributed to the faster CKD progression in African Americans compared with Caucasians. This effect was observed regardless of whether participants had diabetes. This was the first report that showed *APOL1* gene variants not only increase risk for CKD, but also affect kidney disease progression across the broad spectrum of CKD.

Higher Urinary Excretion of Sodium Is Associated with Increased Risk of Cardiovascular Disease:

CRIC researchers have reported that higher levels of sodium in the urine are associated with increased risk of CVD. The analysis included the collection and analysis of urine samples and the CVD outcomes that ensued. CVD outcomes included congestive heart failure, stroke (death of brain cells due to inadequate flow of oxygen-rich blood to a portion of the brain), or heart attack.

The landmark CRIC study has provided invaluable insights into CKD risk and progression (and associated cardiovascular disease) in the United States. Its findings 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 risk factors for CKD and progression to kidney failure.

The CRIC study is one of the largest and longest ongoing studies of CKD epidemiology in the United States. It is a collaboration among 13 U.S. clinical sites—Case Western Reserve University, Cleveland; the Cleveland Clinic, Cleveland; The Johns Hopkins Medical Institutions, Baltimore; Kaiser Permanente Northern California, Oakland; MetroHealth Cleveland, Cleveland; St. Johns Medical Center, Detroit; Tulane University, New Orleans; University of California San Francisco, San Francisco; University of Illinois, Chicago; University of Maryland, Baltimore; University of Michigan, Ann Arbor; University of Pennsylvania, Philadelphia; and Wayne State University, Detroit. The study's Scientific and Data Coordinating Center is at the University of Pennsylvania.

Delineating the Anatomy of the Developing Genitourinary Tract

The genitourinary (GU) tract is the organ system (kidney, ureter, bladder, urethra, prostate, testis, epididymis, vas deferens, penis, ovary, uterus, and vagina) most commonly affected by inherited birth defects, and many reports suggest that some of these birth defects are increasing. Thus, investigations defining normal embryonic development of the GU tract have significant clinical relevance, given the significant health impact of inherited as well as acquired diseases of this organ system. Understanding normal development can help researchers gain insights into what processes go awry in disease, and can lead to the design of therapies. Until recently, GU developmental research was limited by lack of cell-specific markers for key cell lineages (e.g., podocytes, parietal cells, and mesangial cells), incomplete understanding of the normal cellular structure of the major organs of the GU tract, and the lack of a detailed integrative database to understand complex data linked to developmental processes during specific locations in time and space.

The GenitoUrinary Development Molecular Anatomy Project (GUDMAP)

In 2004, the NIDDK and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) established the GUDMAP consortium to begin efforts to identify the cell types that comprise the developing organs of the GU tract and their locations and to generate tools to facilitate research. The major goal of this discovery science effort is to provide the scientific and medical community with a searchable molecular and cellular atlas and cell-specific transcriptional profiles (whether genes are “on” or “off”) to facilitate research. Examples of GUDMAP data include an atlas of gene

expression in the developing mouse and human kidney, and illustrative anatomical subcompartments of the developing mouse lower GU tract including the associated male and female reproductive structures.

In addition to providing tools to the research community, GUDMAP investigators have made significant scientific contributions. For example, a recent study identified a potential role of the Six2 protein in the species differences between mice and humans in determining the duration of kidney development and the final nephron (functional unit of the kidney) number in the mature kidney. This will allow investigators to model and understand human development better.

Investigator-initiated Research

The Institute also supports a robust and productive investigator-initiated research program that includes studies into repair mechanisms and translational studies. Highlights include the following research advances.

Proteins FGF9 and FGF20 have been shown to be both necessary and sufficient for the maintenance of early stage (progenitor) cells that mature into the mouse nephron. Understanding the factors that regulate the size of the pool of nephron progenitor cells that gives rise to nephrons is important, as lower nephron numbers predispose an organism to higher risk of hypertension and kidney disease. In addition, investigators have identified a second pool of self-renewing progenitor cells that gives rise to the stromal tissue—the connective tissue of the kidney—and contributes to the nephron progenitor cell pool.

As progenitor cells mature into the specialized cells of the nephron, the pool of nephron progenitor cells

STORY OF DISCOVERY

decreases and the remaining “older” progenitor cells have a reduced capacity to self-renew (proliferate) and mature more quickly into nephrons. Investigators have shown that “older” progenitors can be rejuvenated when brought into contact with “younger” progenitors, thereby increasing the number of progenitor cells within the pool. These new findings indicate that progenitor cells are not “locked” into an aging process and provide a strategy to increase or replace the progenitors that mature into nephrons.

For the first time, investigators have developed laboratory-based procedures to isolate and expand numbers of mouse nephron progenitor cells by promoting their self-renewal, as well as to direct them towards a mature cell type. This “synthetic” niche sets the stage for studies of nephron development with the ultimate goal of tissue repair/replacement. Researchers reported that kidney mesenchymal-like stem cells isolated from the adult mouse kidney collecting duct can self-renew in the laboratory and, when injected into the mouse kidney, integrate back into the collecting duct.

Using information from GUDMAP that both the *Vangl2* mRNA and *Celsr1* mRNA are present in early-stage mouse kidney development, other investigators have shown that these two genes are necessary for kidney growth and proper maturation.

Taken together, these findings greatly advance knowledge of kidney development in the mouse.

Ongoing Consortia Efforts

Building upon these foundational research findings, the NIDDK is now supporting the following consortia to advance research progress through collective efforts.

- The Human GUDMAP (hGUDMAP) consortium builds upon the existing database and website infrastructure, and retains the basic long-term objectives of GUDMAP—the establishment of a comprehensive understanding of human kidney and urinary tract tissue development to inform the study of tissue maturation and aging, defective organ development, and changes that occur in disease.
- The (Re)Building a Kidney (RBK) consortium aims to develop new therapeutic options for kidney failure, including strategies to repair injured kidneys in the body or generate functional replacement tissue in the laboratory for transplant.
- New knowledge acquired through hGUDMAP and RBK, including the development of technologies and methods of studying tissue, will help jumpstart the Kidney Precision Medicine Project (KPMP). The KPMP aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury or chronic kidney disease, create a kidney tissue atlas, define disease subgroups, and identify critical cells, pathways, and targets for novel therapies.

Dr. Joseph Bonventre— Kidney Repair and Regeneration Shop

Dr. Joseph Bonventre is Samuel A. Levine Professor of Medicine at Harvard Medical School, Boston. He is also Chief of the Division of Renal Medicine and the Chief of the Division of Engineering in Medicine at Brigham and Women's Hospital. Dr. Bonventre earned his B.S. from Cornell University, followed by his M.D. from Harvard Medical School and Ph.D. in biophysics from Harvard University. After his medical internship and residency at Massachusetts General Hospital (MGH), Dr. Bonventre completed a clinical nephrology fellowship at MGH. He is a widely recognized scientist, clinician, and teacher, known for his research in various aspects of the cellular injury and repair mechanisms in the kidney. Dr. Bonventre has been recognized by his peers for numerous accomplishments, including elected membership to the American Society of Clinical Investigation, the Association of American Physicians, and the American Institute for Medical and Biological Engineering. He has been awarded the Osler Medal of the Royal Society of Physicians and the Bywaters Award from the International Society of Nephrology for his contributions to the field of acute kidney injury. Dr. Bonventre currently serves as Editor of Seminars in Nephrology and is on the Editorial Board of a number of journals. The NIDDK has supported his research for more than 30 years. At the January 2016 meeting of the NIDDK Advisory Council, Dr. Bonventre presented his laboratory's recent research findings. The following are highlights from his presentation.

Dr. Bonventre has a longstanding interest in various aspects of the cellular injury and repair mechanisms in the kidney, with a special emphasis on the role of inflammation, biomarkers, and stem cells.

Chronically impaired kidney function—also called chronic kidney disease (CKD)—often leads to end-stage kidney disease. CKD results in dysregulation of many body systems and is a major risk factor for cardiovascular disease. A considerable public health concern as well as a financial problem for the United States, CKD is increasingly important globally. This is partially driven by the growing epidemic of diabetes, of which CKD is a major side effect.

Over the past few years an underlying theme has emerged—a close relationship between CKD and acute kidney injury (AKI). In contrast to CKD, which usually progresses slowly over time, AKI is characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. Dr. Bonventre pointed out that AKI leads to CKD, and CKD clearly predisposes to AKI. Additional research is needed to understand the mechanisms of this relationship, and to learn how to prevent AKI and prevent development and/or progression of CKD after AKI.

Kidney Repair and Regeneration

AKI may arise from a number of causes, such as sepsis (a serious, whole-body inflammatory reaction usually caused by infection), decreased perfusion of blood, or kidney damage from drugs or toxins. AKI is associated with high in-hospital mortality rates. Even though most people with AKI who survive will regain some degree of kidney function, many do not. There is no effective drug therapy to mitigate or reverse AKI. Dr. Bonventre explained that under normal conditions the kidney tubule, made up of intact epithelial cells, functions by reabsorbing water and salts. When the mouse kidney proximal tubule is experimentally injured, the epithelial cells can be lost or damaged.

SCIENTIFIC PRESENTATION

Damaged epithelial cells lose their polarity—meaning that proteins that are assigned to be in one part of the cell become randomly dispersed. When the healthy kidney proximal tubule initially sustains an injury, several tissue and cellular responses can occur, including the death of epithelial cells that form the renal tubules, and injury to blood vessels resulting in stickiness of the endothelial cells that line the capillaries. White blood cells (called leukocytes) adhere to the cells and move across the damaged endothelium. Filtrate is blocked as it flows through the tubule, and there is an increased number of immune cells called “M1” macrophages that migrate into the kidney. Following injury, the tissue begins a process to repair itself. The tissue repair process can be “adaptive” or “maladaptive,” depending on factors such as the severity of the injury and the age of the organ.

In adaptive tissue repair, the repair process is successful, and a fully functional kidney is restored. Several cellular responses contribute to adaptive repair in the kidney, including the presence of immune cells called “M2” macrophages, which support an increase in the number of epithelial cells from pre-existing epithelial cells remaining in the tubule, reduced numbers of inflammatory cells, and an increase in the number of endothelial cells. Dr. Bonventre explained that his lab discovered that new epithelial cells derived from pre-existing epithelial cells during repair. These results were obtained from lineage-tracing studies—where tubule cells are biologically

labeled, and daughter cells identified by the persistent presence of the label.

Maladaptive tissue repair is characterized by persistent inflammation, and an increased number of myofibroblasts, which are responsible for an increased deposition of extracellular matrix (also referred to as fibrosis) with corresponding organ dysfunction. To better understand the pathophysiology of kidney injury, a new mouse model of acute kidney injury was used to study the proximal tubules. The tubules reabsorb about two-thirds of the fluid filtered by the glomeruli, the filtering units of the kidney's nephrons. After inducing a one-time injury in a specific region of these tubules, Dr. Bonventre and his

Two normal adult healthy kidneys can filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Each kidney is made up of about a million filtering units called nephrons, although the exact number varies widely among individuals. The nephron includes a filter, called the glomerulus, and a complex tubule. The nephrons work through a two-step process. The glomerulus lets fluid and waste products pass through it; however, it prevents blood cells and large proteins from passing. The filtered fluid then passes through the tubule, which has multiple segments that send needed minerals back to the bloodstream and remove wastes. The final product becomes urine.

colleagues observed severe tubular injury, along with the proliferation of tubular epithelial cells and the appearance of inflammatory cells. Following this single injury, the kidney recovered completely. However, when the researchers induced three injuries at 1-week intervals, they observed diminished cellular repair, with significantly increased kidney tissue fibrosis both in the glomerulus (glomerulosclerosis) and in the area of the tubules, as well as leakage of protein into the urine (proteinuria). The kidney tubule was unable to repair itself after three successive injuries, and a chronic disease process ensued.

These findings have been extrapolated to other situations, for example, in studies using the “Akita” mouse model of type 1 diabetes. After inducing a one-time kidney injury in these diabetic mice,

SCIENTIFIC PRESENTATION

there is increased kidney fibrosis, proteinuria, and glomerulosclerosis, indicating progressive chronic kidney disease. When the researchers induced three injuries (one per week) to the proximal tubule at 1-week intervals, the consequences were so severe that the animals did not survive. From these results, Dr. Bonventre commented that animals with pre-existing conditions, such as diabetes or hypertension, may be much more limited in their ability to repair an insult to the kidney when compared to animals with no underlying diseases.

Dr. Bonventre further examined the state of kidney cells just after injury, to see whether or not the cells were replicating. Cells replicate themselves through an organized, step-by-step process called the cell cycle, which consists of phases for growth (referred to as G1, S, and G2 phases) and division (M phase). The cycle has checkpoints, which allows the cell to halt the cycle for repairs. Dr. Bonventre's laboratory has provided evidence that injury to tubular epithelial cells, by three different experimental approaches, stalls the cells at the G2/M checkpoint—inhibiting their ability to progress through the cell cycle to produce two daughter cells. Further examination elucidated the existence of a strong correlation between G2/M arrest in tubular cells and fibrosis. For example, the longer the tubular cells remain in the G2/M checkpoint, the greater was the production of molecules that promote fibrosis. Thus, under conditions when the injury is sufficient to prevent the normal process of DNA damage repair, the cells arrest in G2, leading to maladaptive repair. The progression to the maladaptive repair process in these animal model systems resembles the transition from acute kidney injury to chronic kidney disease in humans.

Dr. Bonventre explained that his research team was interested to learn why humans are unable to generate new kidney nephrons after birth, with the hope that such knowledge may lead to strategies to induce kidney nephrons in the body to regenerate

after injury. Fish are capable of regenerating nephrons after birth, but mammals are unable to do this. Rats and mice can generate new nephrons up to 2 to 3 days after birth but it was not clear whether these were nephrons generated *de novo* (starting from the beginning) or nephrons that matured from early committed developmental structures present at birth. In an attempt to gain insight into whether the mouse kidney could be coerced to generate entirely new nephrons after birth, the kidneys of newborn mice were experimentally injured to remove nephrons at the day of birth. The finding, unfortunately, was that injured nephrons were not replaced even if the other kidney was removed at the time of surgery.

Another interest of Dr. Bonventre and his team is the formation of new kidney organoids in the laboratory. A kidney organoid is a three-dimensional, laboratory-grown set of cells that mimics characteristics of normal kidneys. Building on previous knowledge of how the normal kidney develops, the team began their quest to form kidney organoids using human induced pluripotent stem cells (iPSCs). iPSCs are cells that have been experimentally induced to revert to an earlier stage of development (embryonic stem cell-like) and are capable of developing into all the different cell types of the body. By trial and error (adding various compounds in a three-dimensional cell culture system), the iPSCs formed spheroids. By inhibiting a protein called glycogen synthase kinase 3 β and adding growth factors in specific sequences, the spheroids developed into kidney organoids—possessing tubule- and glomerulus-like structures. To test whether the kidney organoids respond similarly to injury as do actual kidneys, the organoids were treated with a toxic compound (cisplatin) often used in cancer chemotherapy in humans and then assessed for injury by expression of the clinical biomarker kidney injury molecule-1 (KIM-1) previously discovered and characterized by Dr. Bonventre. As would be the case with

SCIENTIFIC PRESENTATION

actual kidneys, the tubule-like structures within the organoid that expressed characteristics of the proximal tubule produced KIM-1 upon injury with low levels of cisplatin, whereas uninjured spheroids did not, indicating the response was specific to injured kidney organoids.

The kidney organoids were further investigated to determine their potential to serve as a functional model of polycystic kidney disease (PKD). PKD is a genetic disorder that causes numerous cysts to grow in the kidneys. Kidney cysts are abnormal sacs filled with fluid that result in chronic kidney disease (reduced kidney function over time). Using a gene-editing technology called CRISPR/Cas9, genetic mutations were introduced into the genes known to contribute strongly to PKD cyst formation in iPSCs, and then the

cells were subjected to the process to form organoids. Organoids containing mutations in PKD cyst-forming genes, that were exposed to a compound that is known to increase cellular cAMP, formed balloon-like, fluid-filled sacs that appear to model the human disease. This finding suggests that these genetically mutated organoids may serve as a faster way to screen drugs for potential therapeutic use in humans.

Future Directions

Dr. Bonventre closed by thanking his research team for their contributions. His research efforts on repair and rebuilding or replacing the kidney serve as a foundation on which to increase our knowledge to ultimately develop new effective therapies for people with kidney disease.

Paying It Forward—A Physician-scientist with Type 1 Diabetes Participates in a Clinical Trial To Prevent Kidney Disease



Dr. Robert Truckner

As a pediatric emergency physician and research scientist who has type 1 diabetes, Robert Truckner has personally witnessed, as both a patient and a doctor, the remarkable technological advances made over the past few decades that have improved the lives of people with type 1 diabetes. However, he has been concerned about developing kidney disease and other diabetes-related complications since shortly after he was diagnosed with type 1 diabetes as a child in the 1970s. “You’re worried about kidneys,” he says, as well as other organs that could be adversely affected by type 1 diabetes, such as the eyes and the heart. These fears “have always been in my mind,” Robert remembers. Although rates of complications are lower now than they

“You’re worried about kidneys,” he says, as well as other organs that could be adversely affected by type 1 diabetes, such as the eyes and the heart. These fears “have always been in my mind,” Robert remembers.

were when Robert was growing up, kidney disease remains a common complication of type 1 diabetes. After years of managing his own diabetes, Robert, now in his mid-50s, helps counsel his own patients with type 1 diabetes when they arrive at the emergency room, often for diabetic ketoacidosis—an acute and dangerous complication of diabetes. His personal experience with type 1 diabetes and its complications allows him to connect with and reassure his adolescent patients in a way that may not be possible for many other physicians. Robert laughs as he recalls the times he has told his young patients: “I’ve been doing this [managing type 1 diabetes] for four times longer than you’ve been alive.”

Living with Type 1 Diabetes, and the Fear of Kidney Disease and Other Complications

Robert was diagnosed with type 1 diabetes when he was about 10 years old. He recalls one year around Christmas, his observant mother noticed that he was excessively thirsty and hungry, and was losing weight—characteristics that he later learned were

classic signs of type 1 diabetes. After visiting his equally sharp family doctor, followed by a trip to the hospital, Robert

and his family were told the difficult news that he had type 1 diabetes. Because Robert was so young, it took some time for him to fully understand what this diagnosis meant. But soon he began to realize how managing his health would change his life. He

PATIENT PROFILE

learned to give himself insulin shots, to monitor his urine glucose levels, and to help maintain his health by living a very active lifestyle.

Type 1 diabetes presented a variety of challenges throughout life. As a child, Robert spent years trying diligently to control his blood sugar as best as was possible at the time, but without the benefit of a robust community nearby to provide support. "It was a very isolating experience," Robert remembers; "I didn't know anybody who had diabetes—no one."

But that changed when he was 19 years old, and

he began working at

Camp Midicha—a

summer camp run

by the Michigan

Children's Diabetes

Association—where

he interacted with kids

ages 6 to 16 who had diabetes. Initially he was a counselor, and later became its Director for a couple of years. "This was mind-opening for me," says Robert of his time working with these youths at Camp Midicha, "just a phenomenal experience."

Robert remembers always planning a career in medicine in the back of his mind. As a young teenager, he wrote a letter to a prominent diabetes center asking a physician there for advice about whether he should become a physician himself, considering his diabetes. He remembers the response: "I got a letter back ... but they discouraged me, really, from becoming a physician," noting that the hours are long and will take a toll on his health. While the letter was somewhat dispiriting, Robert credits his parents with instilling in him the perseverance to pursue his dreams. This was a value that "my Mom and Dad ingrained in me from a very young age ... that there was nothing I couldn't do, even with diabetes." Heeding this advice, Robert applied and was accepted to medical school. A few years later, he again faced an important professional decision about which area of

specialization to pursue—a choice that he admits was influenced by the possibility of complications from diabetes. "I really liked orthopedic surgery. But I chose not to go into it because I was worried if I got retinopathy at some point, I would not be able to perform." (Diabetic retinopathy, a type of diabetic eye disease, can lead to severe vision loss and blindness.) "That was one time that ... I let the fear of diabetes take control of me, the fear of the unknown of diabetes." However, he says cheerfully that he has been very happy with his chosen career of pediatric emergency medicine. Now

Robert credits his parents with instilling in him the perseverance to pursue his dreams. This was a value that "my Mom and Dad ingrained in me from a very young age ... that there was nothing I couldn't do, even with diabetes."

Robert helps his young patients who have type 1 diabetes learn to manage their health better. He offers them hope for a brighter future by reminding

them that over the past few decades, technological advances have greatly improved his own blood sugar control and management of complications. He often tells them: "The technology in my lifetime has been amazing; the technology of your lifetime will be even more amazing."

Even with the new and improved technologies currently available to help Robert and other people with type 1 diabetes manage their disease, it is difficult even for the most vigilant patients to achieve levels of blood sugar control that research has shown can reduce the risk of long-term complications, including kidney disease. Thus, in addition to providing support for studies that have contributed to technology development and improved understanding of the long-term effects of diabetes, NIDDK is also funding research to identify other approaches to prevent and treat diabetes-related complications. Toward this goal, in fall 2015, Robert enrolled in an NIDDK-funded study investigating whether an inexpensive drug, called allopurinol, can help slow the decline of kidney function in people with type 1 diabetes and very early kidney damage.

PATIENT PROFILE

Diabetic Kidney Disease and the PERL Clinical Trial

Diabetes is the most common cause of kidney disease and can lead to kidney failure, requiring dialysis or kidney transplantation—both highly invasive treatments. A growing number of research studies suggest that reducing the level of the bodily waste product uric acid in the blood could help curb the deterioration of kidney function. The drug allopurinol has been used for decades to lower uric acid levels in patients with gout, which is a painful condition that occurs when uric acid is deposited as needle-like crystals in the joints and/or soft tissues. Allopurinol exhibits an excellent safety profile and is a generic, relatively inexpensive drug—attractive characteristics for a potential preventative therapy for a prevalent condition.

The NIDDK-supported Preventing Early Renal Function Loss in Diabetes (PERL) clinical trial is investigating whether allopurinol can prevent or delay the loss of kidney function in people with type 1 diabetes and very early kidney damage. Several centers around the United States and Canada have enrolled patients so populations from different geographic locations will be included in the study. The primary outcome measurement for the study is glomerular filtration rate (GFR)—the rate at which kidneys filter wastes and extra fluid from the blood, serving as a measure of kidney function.

Patients enrolled in the trial, including Robert, have relatively high serum uric acid levels but only mildly or moderately decreased renal function. PERL is a double-blind randomized clinical trial, which means that study participants receive either allopurinol or a placebo control, but neither the participants nor the

scientists who interact directly with them know which patient receives which treatment through the course of the study. Thus, because the trial is still ongoing, Robert does not yet know whether he is receiving allopurinol or placebo. Trial participants receive the treatment for 3 years, after which their GFR levels, as well as other conditions, will be compared to see whether there are differences in health between those who received the different treatments.

A Unique Perspective: Patient, Physician, and Scientist

As a physician and scientist who has been involved in conducting clinical trials, Robert is well-aware of logistical and other issues participants face when enrolling in clinical research studies. Based on his previous experiences, Robert feels that the PERL trial is not as demanding as some other clinical trials. “As studies go,” he says, “this is a piece of cake.” In addition to taking daily pills (either allopurinol or placebo), participants visit the clinic every few weeks for weight measurement and blood sugar analysis, and for kidney function tests. Due to Robert's schedule as an emergency physician, coordinating

these visits is fairly easy for him, but he acknowledges that “people who work 9-to-5 jobs ... may have more difficulty.” He also

notes that while he lives in Spokane, Washington, near one of the sites where the trial is being conducted, study participants are being recruited from a larger area of the Pacific Northwest, so some people may have to travel longer distances.

Interestingly, through conversations with research staff, Robert was made aware of a potential unexpected benefit of participation in the PERL study for some people. He learned that by simply engaging frequently with the well-trained staff, some

“There’s this whole generation of kids” growing up with the fear of developing kidney disease, and “if I can use ... an old medication to save my kidneys,” he ponders, or “to at least study if it does, and it works—wouldn’t that be wonderful?”

PATIENT PROFILE

participants were given valuable advice in passing, such as tips on making healthy dietary choices, that they might not have otherwise received. “I think a secondary effect of these studies for people [who have poorly controlled blood glucose levels],” he says, “is that they get some diabetes education along the way.” In this way, study nurses and others help improve diabetes care for some study participants.

Hope Through Research

Robert recognizes that improvements in technology—made possible through scientific research—have led to improved quality of care for people with type 1 diabetes over the past few decades. By participating in the PERL trial, he will be a part of the ongoing research efforts to develop the next potential wave of life-improving or life-saving

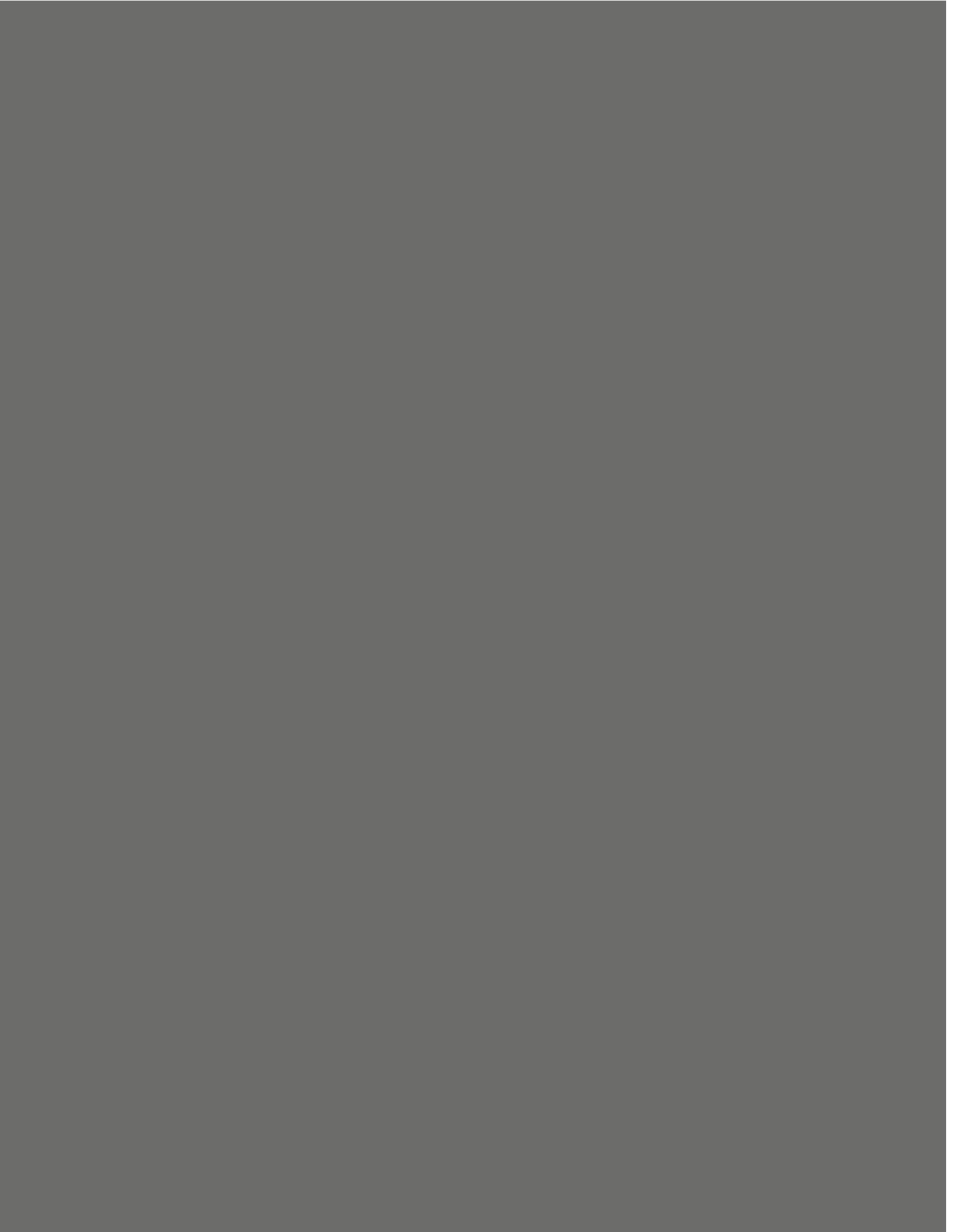
therapeutics. The idea of preventing kidney disease with allopurinol gives Robert hope for all people with type 1 diabetes, but particularly for today’s children. “There’s this whole generation of kids” growing up

with the fear of developing kidney disease, and “if I can use ... an old medication to save my kidneys,”

he ponders, or “to at least study if it does, and it works—wouldn’t that be wonderful?”

From his varied experiences, Robert knows that clinical trials aimed at addressing important health issues, such as preventing kidney disease, require commitment on many levels, by many groups of interested people. Robert expresses great appreciation to all those involved. “I want to say thank you ... thank you to the scientists. I want to say thank you to the Congress. I want to say thank you to NIDDK, and the other study participants,” he reflects. “Thank you for caring.”

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Extramural Funding Trends and Support of Core Values

The 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.

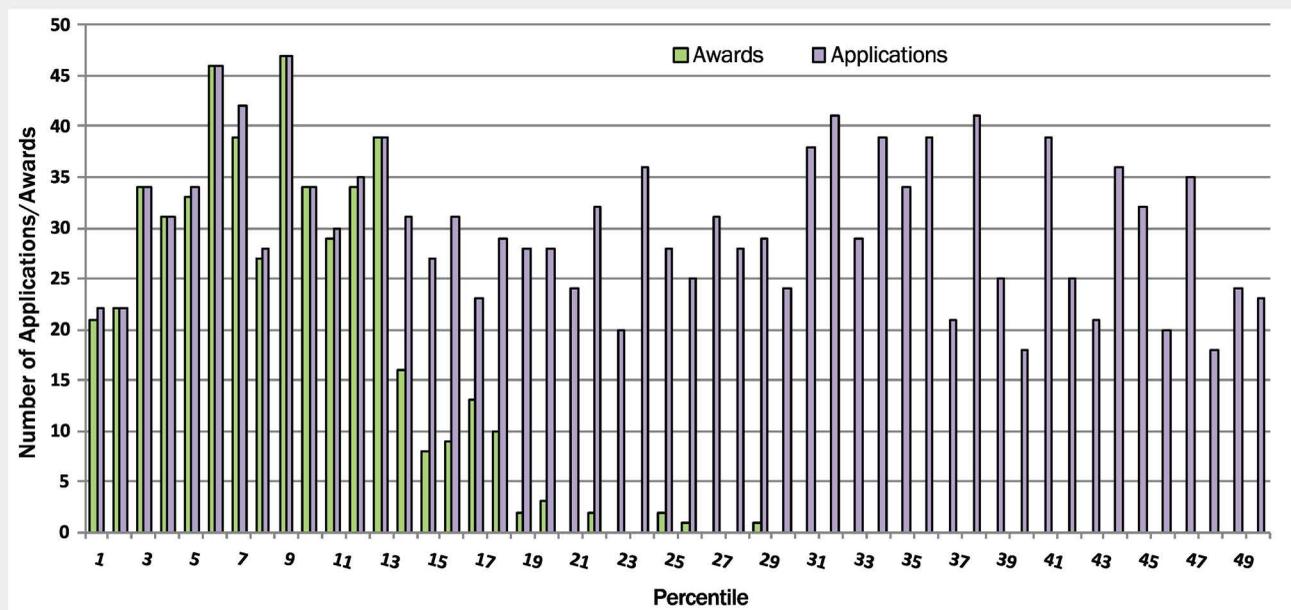
Following that presentation, the NIDDK generated additional data on application and funding trends to help our research community understand application and funding dynamics over recent years and demonstrate the NIDDK's commitment to research and programs associated with the NIDDK's core values and posted these data on the NIDDK website. The NIDDK updates the charts on its website annually; the data shown here were recently updated to include Fiscal Year (FY) 2016.

NIDDK Funding Outcomes for Fiscal Year 2016 and Historical Application and Funding Trends

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

FIGURE 1

FIGURE 1: NUMBER OF NIDDK COMPETING R01 APPLICATIONS SCORING WITHIN THE TOP 50TH PERCENTILE AND NUMBER OF NIDDK PERCENTILED R01 APPLICATIONS FUNDED IN FY 2016

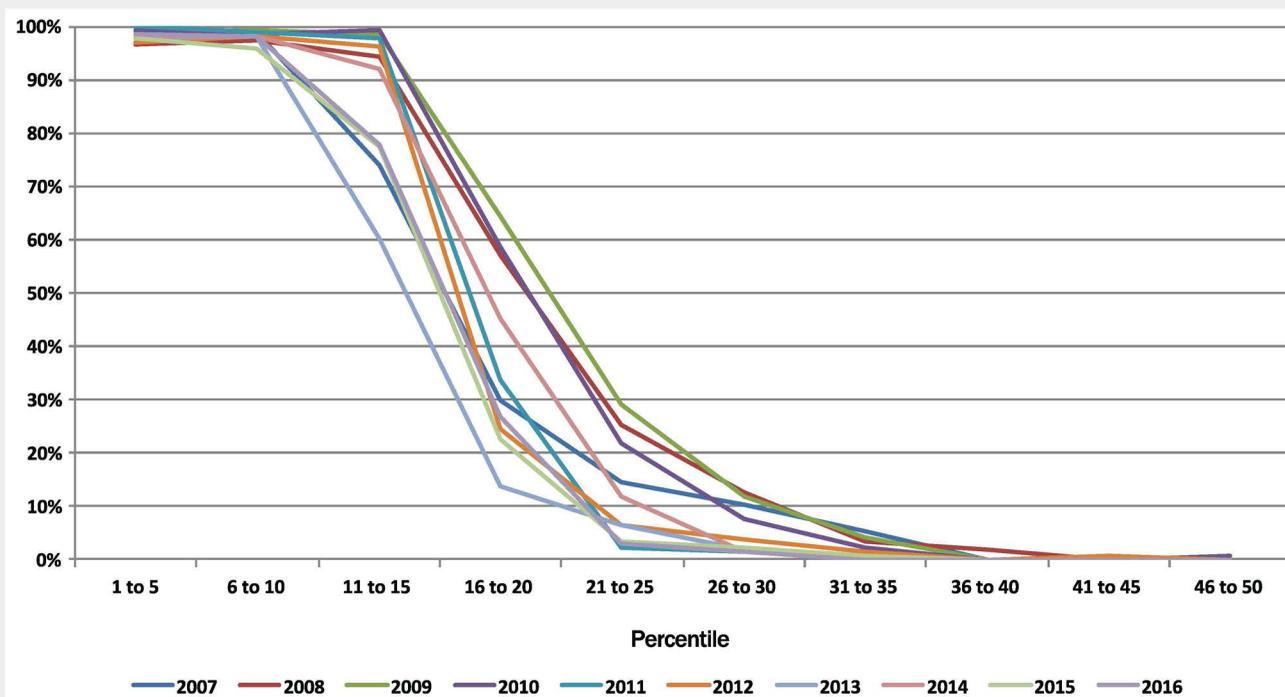


Note: “Applications” shown in the chart above include all applications that scored 50th percentile or better. Unscored applications, scored applications with no percentiles, and applications scoring above the 50th percentile are not shown (49 percent [n=1,433] of the applications received were unscored, scored but did not receive a percentile, or scored above the 50th percentile). No unscored applications were funded in FY 2016.

The NIDDK nominal payline in FY 2016 was the 13th percentile for established investigators and the 18th percentile for Early Stage Investigators (ESIs). The payline and additional programmatic scrutiny for R01 applications requesting more than \$500,000 in direct costs are substantially more stringent. These data show that the NIDDK adheres closely to its payline, but does exercise programmatic discretion to include a limited number of programatically important applications.

FIGURE 2

FIGURE 2: NIDDK COMPETING R01 APPLICATION FUNDING CURVES FOR FY 2007-2016



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

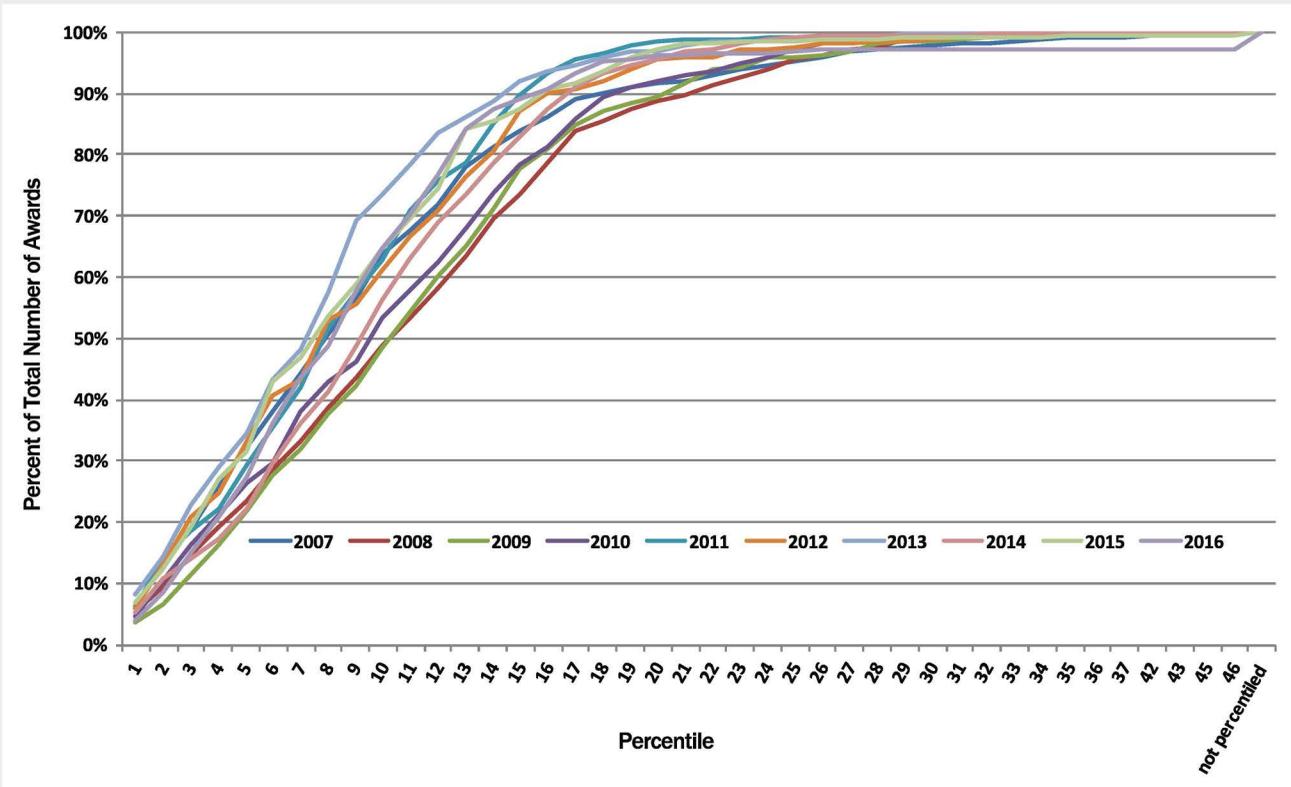
The data demonstrate steep deflections in the percentage of applications funded at or above the nominal payline for each year. The R01 paylines for the years included in Figure 2 are shown in the table to the right.

Fiscal Year	General Payline	\$>500K Payline	New Investigator Payline	Early Stage Investigator (ESI) Payline	ESI First Competitive Renewal Payline
2007	13	8	15	--	--
2008	17	11	19	--	--
2009	17	11	19	--	--
2010	17	11	19	--	--
2011	15	10	17	--	--
2012	13	9	13	18	--
2013	11	7	11	16	--
2014	13	8	13	18	--
2015	13	8	13	18	15
2016	13	8	13	18	15

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

FIGURE 3

FIGURE 3: CUMULATIVE PERCENTAGE OF R01 AWARDS ACROSS PERCENTILES (FY 2007-2016)



Only funded applications are considered in the data set used to generate 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: 0.1-0.9 was summed into 1, 1.1-1.9 was summed into 2, etc. These cumulative funding data again demonstrate that the vast majority of applications funded by the NIDDK fall within the payline, but that the NIDDK does exercise programmatic discretion to include a limited number of programatically important applications. Note that in FY 2016 a limited number of R01 applications in response to specific Funding Opportunity Announcements received a priority score, but not a percentile score. Some of these applications were funded and hence included in this chart. No unscored/streamlined applications were funded in FY 2016.

FIGURE 4

FIGURE 4: NUMBER OF COMPETING NIDDK R01 APPLICATIONS (INCLUDING RESUBMISSIONS) RECEIVED FOR FUNDING CONSIDERATION IN FY 1997-2016

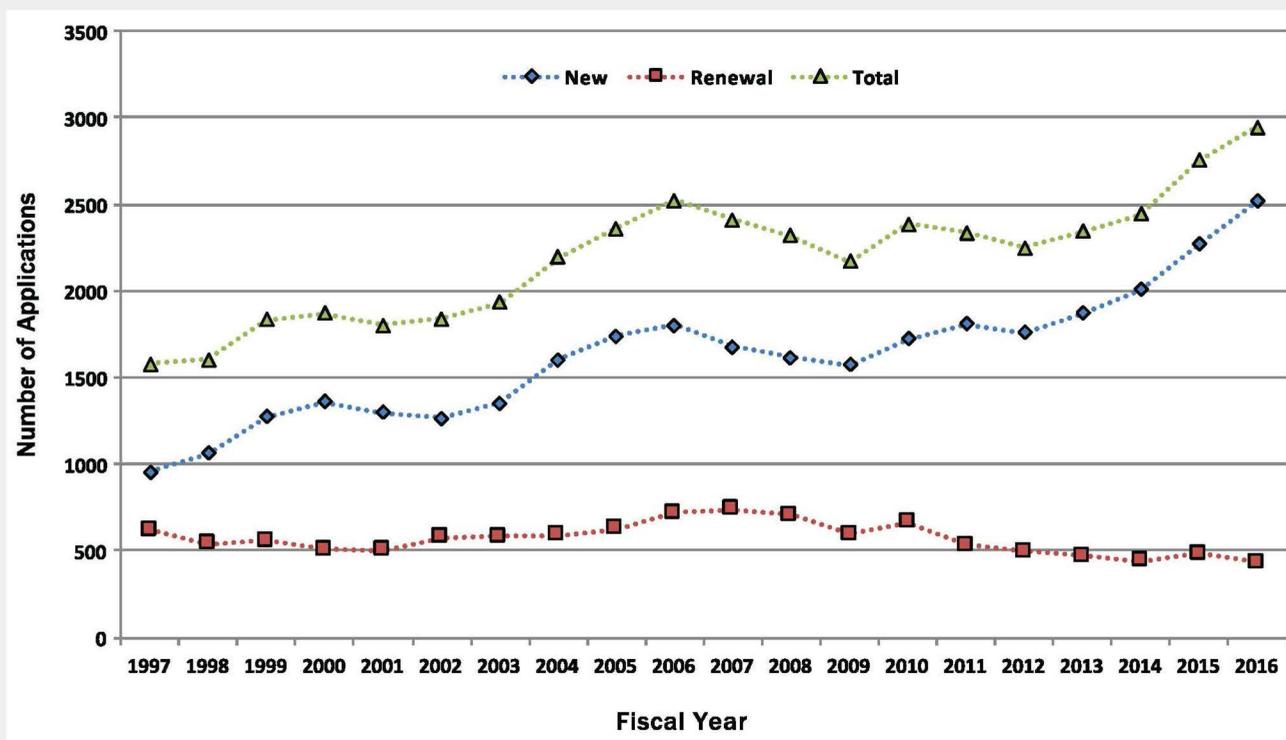
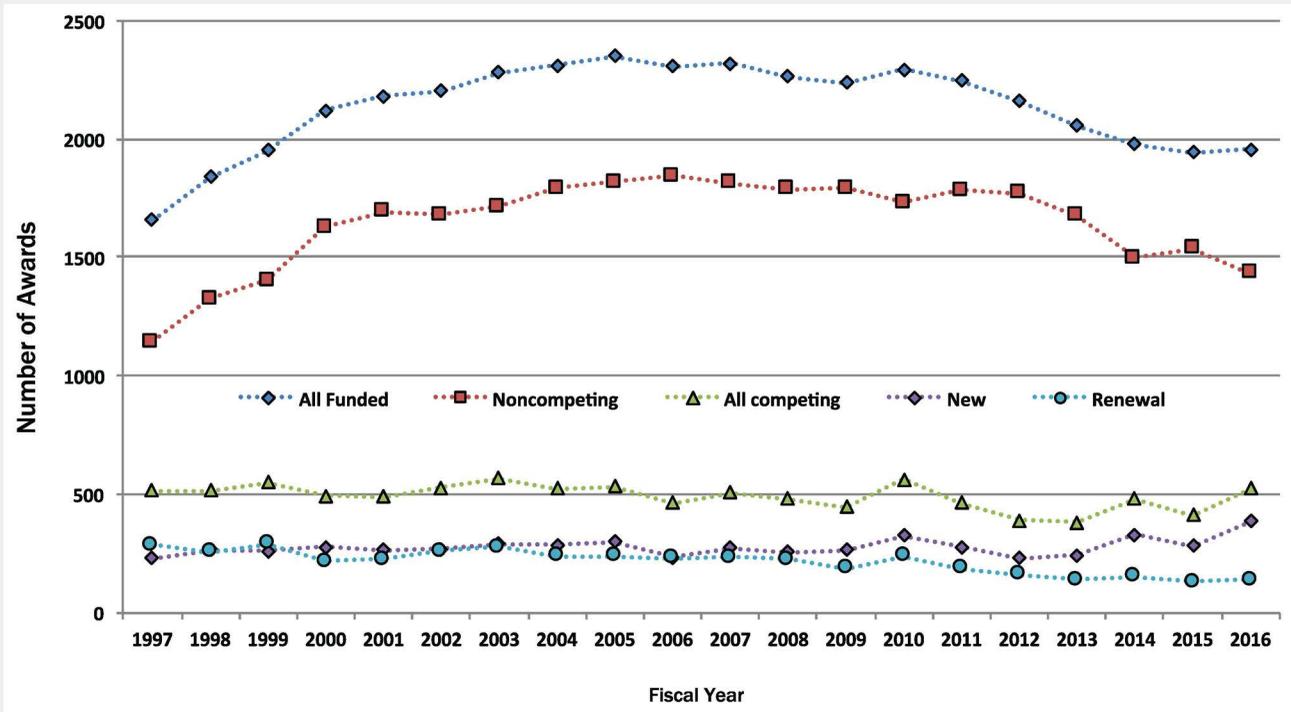


Figure 4 shows a substantial increase in the number of competing R01 applications received by the NIDDK between FYs 1997 and 2016. After some years of relatively flat growth, FYs 2013-2016 have again shown increases. The observed increases between FYs 1997 and 2006 and between FYs 2013 and 2016 were primarily due to increases in the number of new (Type 1) applications. The number of competing renewal applications showed some fluctuation between FYs 1997 and 2016, but overall the number of renewal applications has slightly decreased.

FIGURE 5

FIGURE 5: NUMBER OF NIDDK R01/R37 GRANTS (COMPETING AND NON-COMPETING) FUNDED IN FY 1997-2016



During the doubling of the NIH budget (FYs 1998-2003), the total number of R01/R37 grants funded by the NIDDK increased significantly. After leveling off following the doubling, the number of grants funded by the NIDDK has declined since FY 2007. Prior to FY 2009, slightly fewer than half of the competing grants funded by the NIDDK were new (Type 1) awards in most years. However, since FY 2009 that proportion has risen to 73 percent (in FY 2016).

FIGURE 6

FIGURE 6: OVERALL NIDDK EXPENDITURES (INCLUDES DIRECT AND FACILITIES AND ADMINISTRATIVE COSTS) ON R01 AWARDS (COMPETING AND NON-COMPETING) IN FY 1997-2016

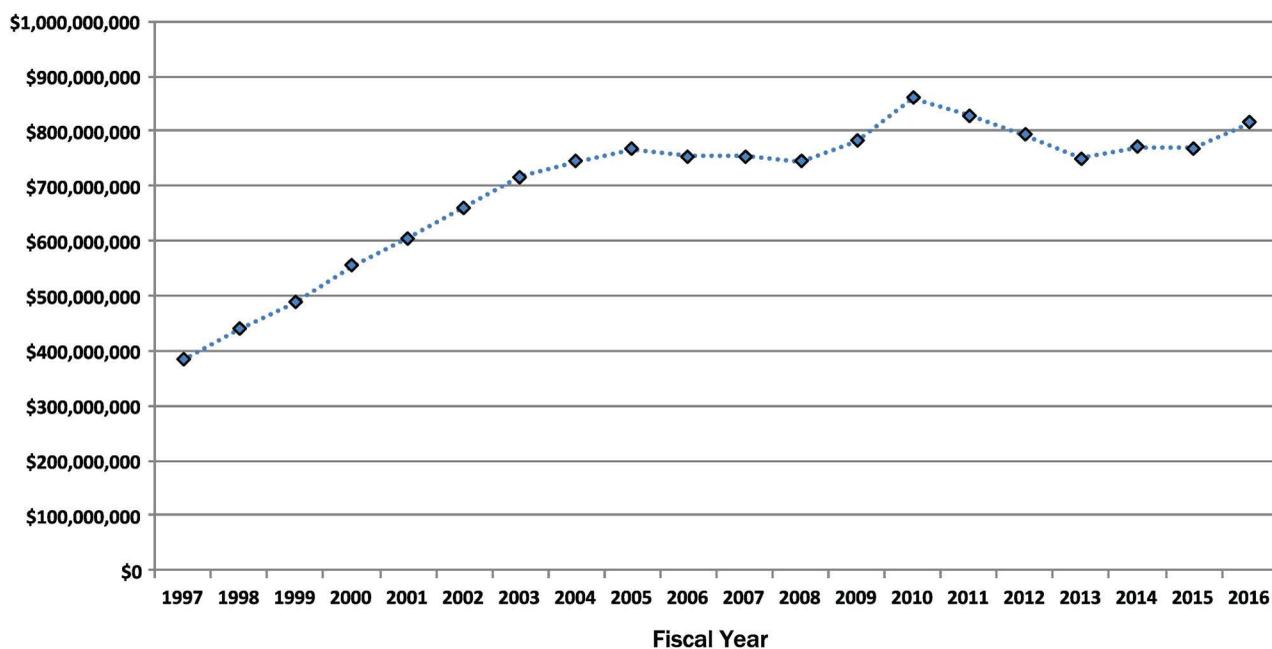


Figure 6 shows that NIDDK expenditures on R01 grants have more than doubled (113 percent increase) since FY 1997. This is because the NIDDK is funding a larger number of these awards (Figure 5), and because the median cost of an R01 has increased substantially (Figure 7).

FIGURE 7

FIGURE 7: MEDIAN TOTAL COSTS (INCLUDES DIRECT AND FACILITIES AND ADMINISTRATIVE COSTS) OF NIDDK R01 GRANTS (COMPETING AND NON-COMPETING) IN FY 1997-2016

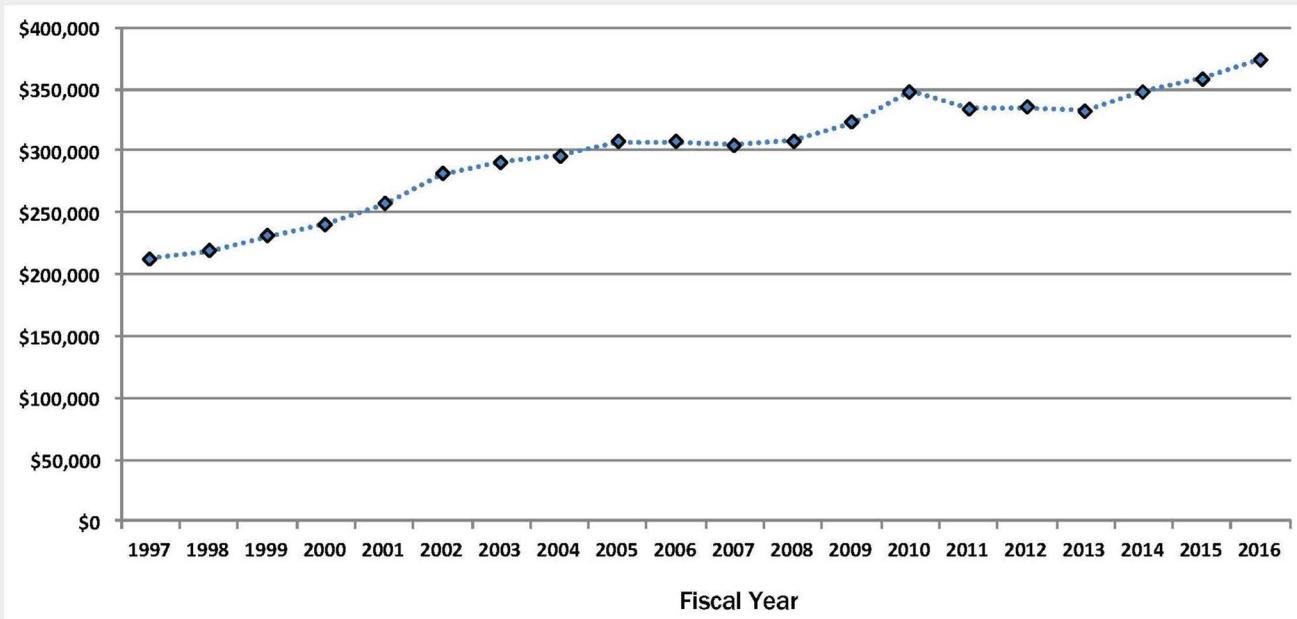


Figure 7 illustrates that the median cost of R01 awards has increased approximately 75 percent since FY 1997.

FIGURE 8

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

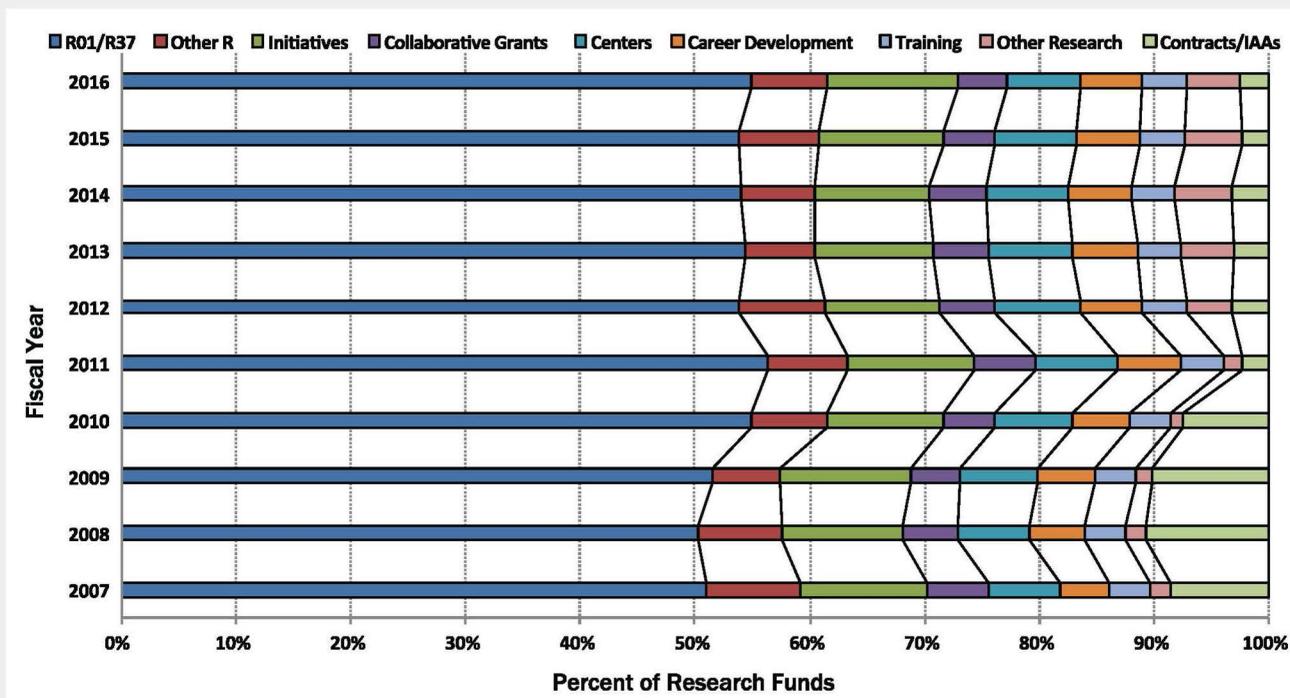


Figure 8 shows that relative funding levels of most NIDDK extramural research categories have remained fairly stable since FY 2007. The original version of these data, encompassing FYs 2003-2011, was presented to the NIDDK’s Advisory Council in May 2012 in the context of the NIDDK’s core values. The 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 (such support is generally represented in the Initiatives and Contracts categories), and continuing strong support of training and career development programs. Figures 9 through 12 illustrate other examples of how the NIDDK’s portfolio has reflected NIDDK core values over time.

NIDDK Portfolio Categories:

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

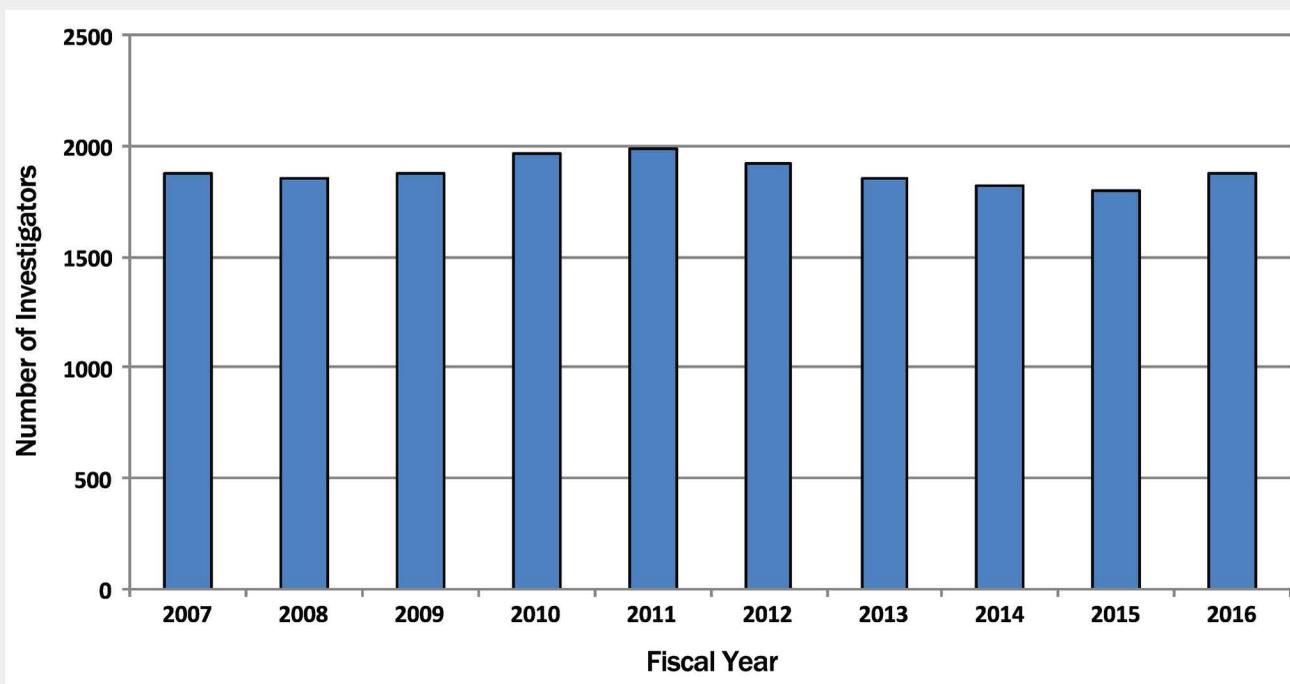


Figure 9 shows that the number of principal investigators (PIs) supported by at least one R01 or R37 remained relatively stable between FYs 2007 and 2016, with slight increases from FYs 2010-2012. It should be noted that in FY 2008 NIH, for the first time, began making multiple principal investigator R01 awards to support team science projects. The observed increases in numbers of PIs supported by the NIDDK immediately following FY 2008 are largely attributable to multiple PI R01 awards. The subsequent changes in numbers of PIs supported by the NIDDK from FY 2012-2016 may, in part, reflect the more stringent paylines during this period, but other factors may also be involved.

FIGURE 10

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

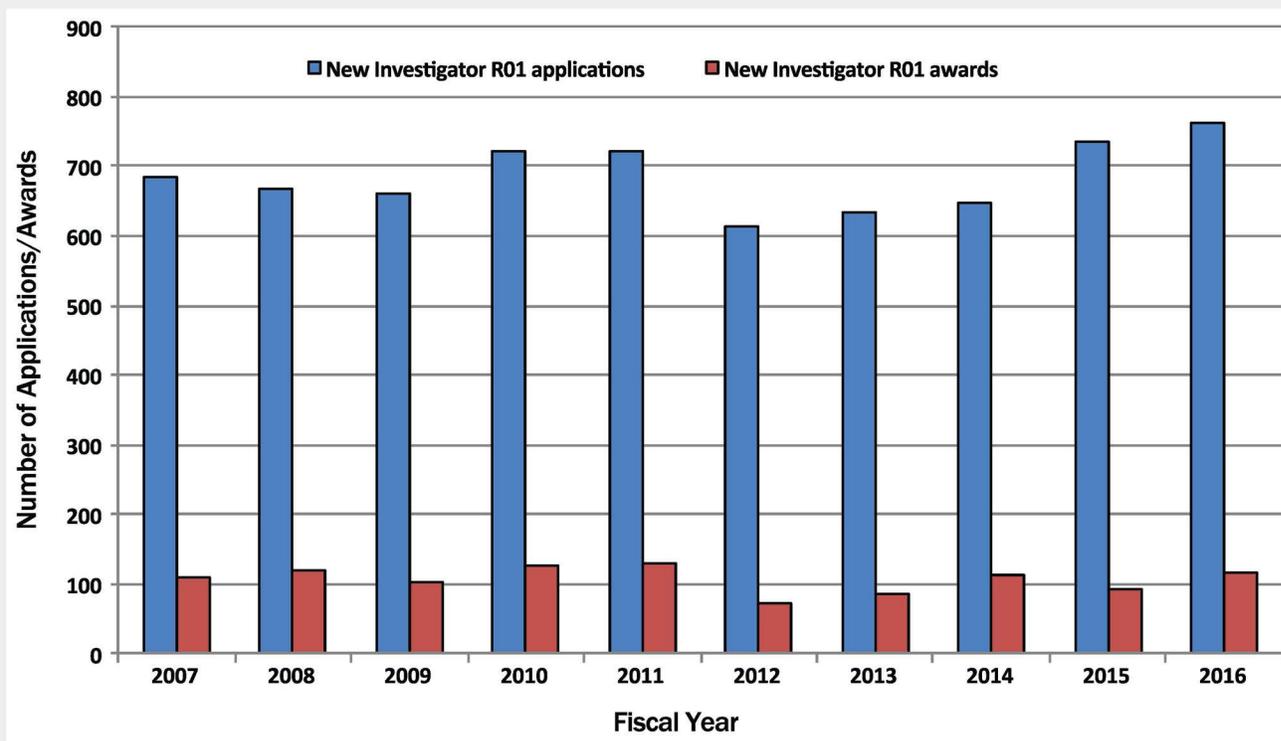
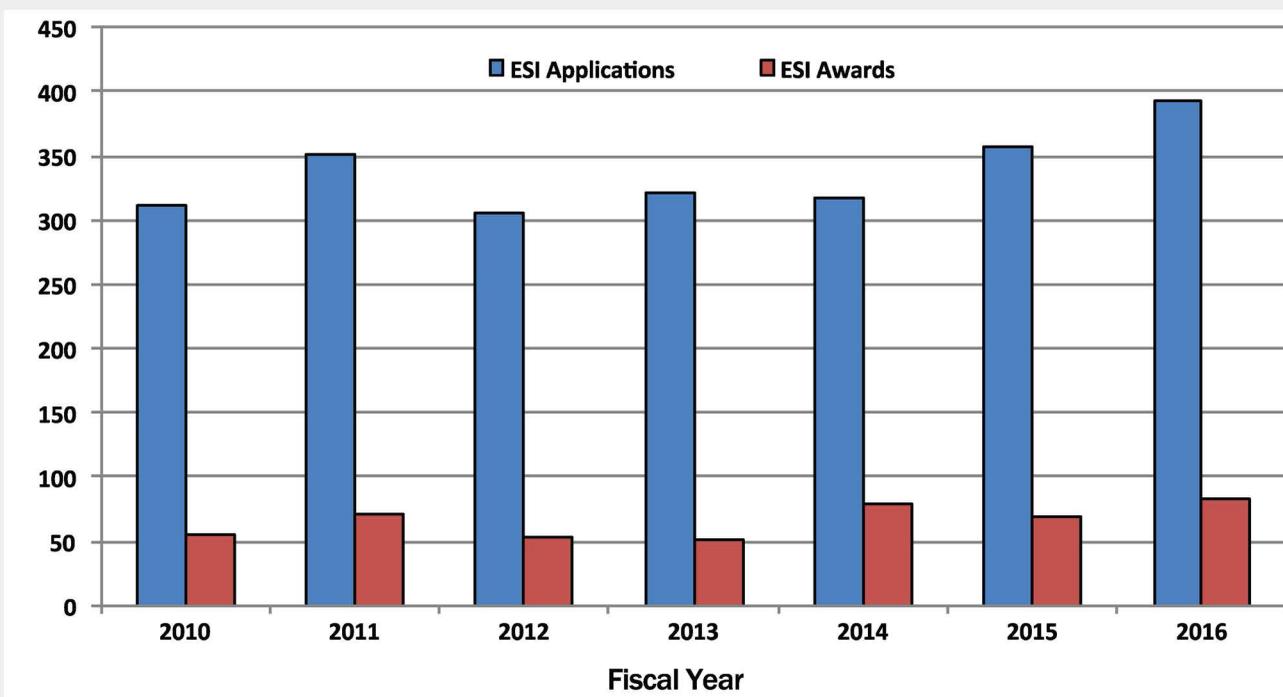


Figure 10: Between FYs 2007 and 2010, the NIH and the NIDDK established new policies focused on New Investigators, and these policies appear effective in mitigating downward pressures on New Investigator awards. After FY 2011 the number of New Investigator applications and awards declined. However, the numbers of New Investigator applications have since recovered and the number of New Investigator awards have fluctuated around about 100 per year. 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 Figures 10 and 11 shows that while 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 table accompanying Figure 12 and the NIDDK New and Early Stage Investigators page at https://www.niddk.nih.gov/research-funding/process/apply/new_early_stage_investigator/Pages/new_early_stage_investigator.aspx). Although there is moderate fluctuation from year to year in the numbers of ESI applications and awards, the differential payline is contributing to a healthy success rate for these applications.

FIGURE 12

FIGURE 12: PRESERVING A STABLE POOL OF NEW INVESTIGATORS—PERCENT OF NIDDK NEW INVESTIGATOR APPLICATIONS SUBMITTED AND AWARDS RECEIVED BY EARLY STAGE INVESTIGATORS (ESI)

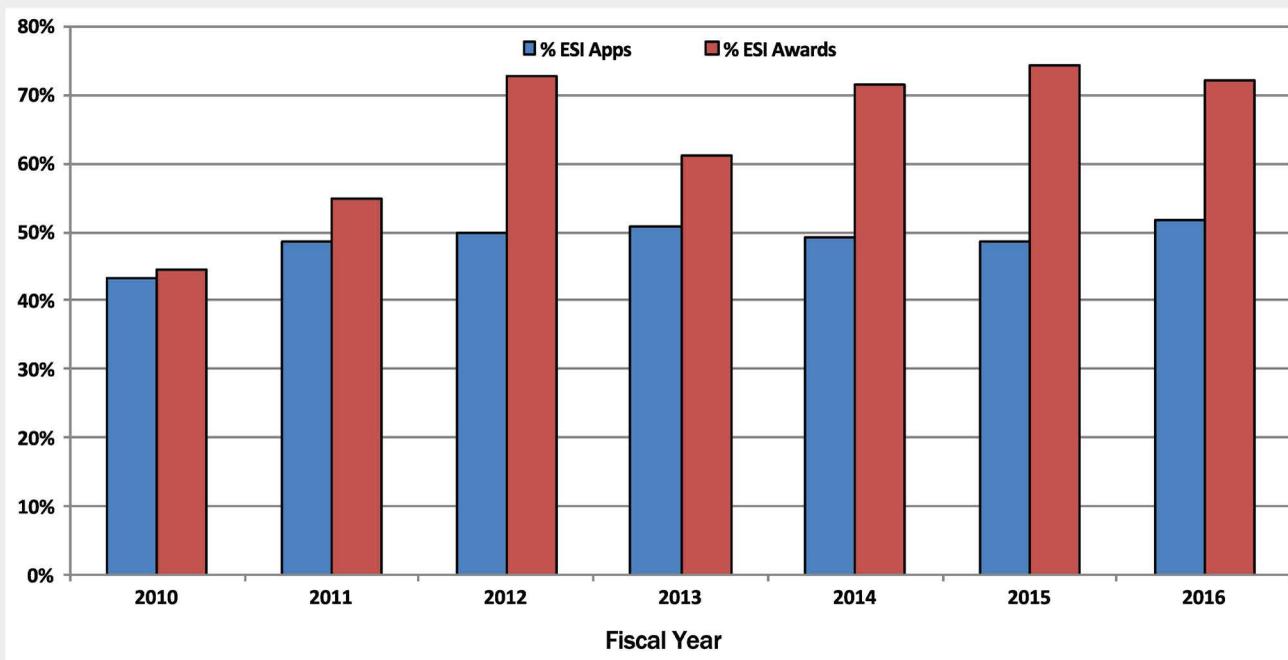
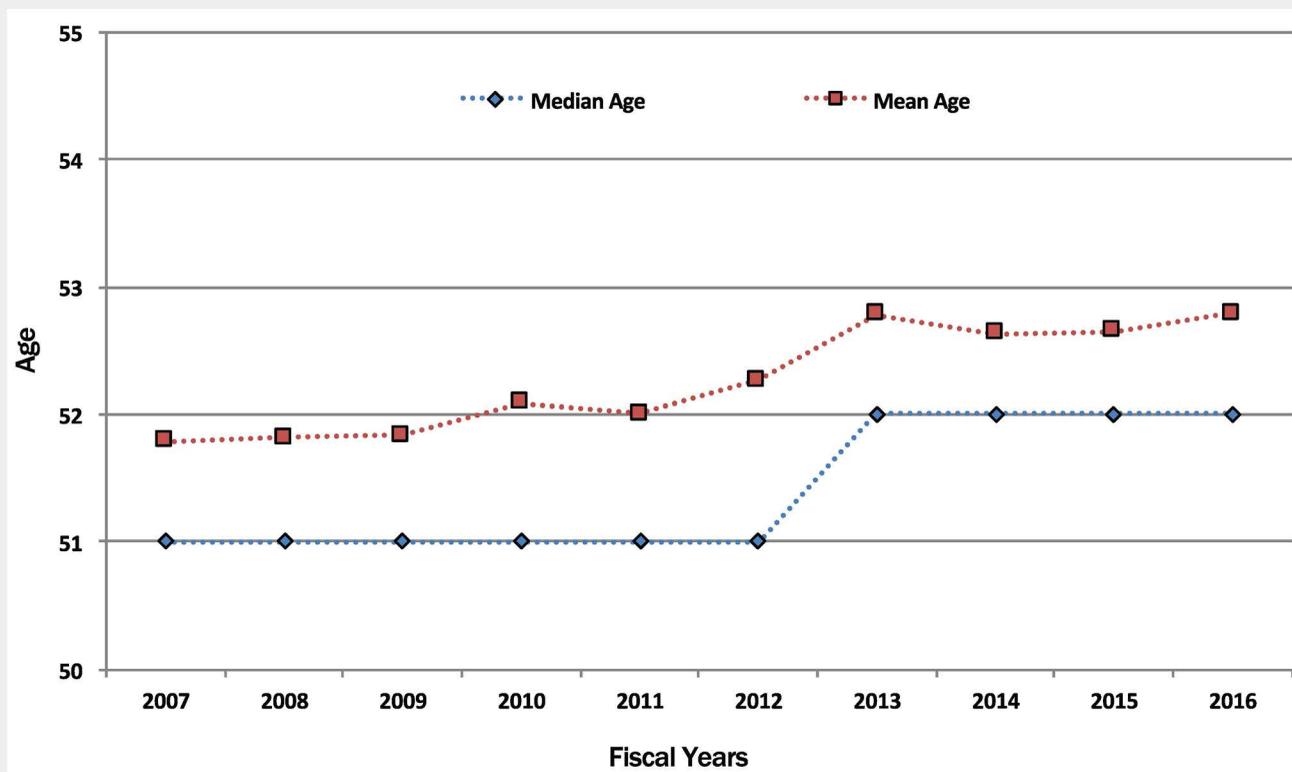


Figure 12 shows that the NIDDK's differential payline for ESIs from FY 2012-2016 (see table accompanying Figure 2 and the NIDDK New and Early Stage Investigators page at https://www.nidk.nih.gov/research-funding/process/apply/new_early_stage_investigator/Pages/new_early_stage_investigator.aspx) has been effective in enhancing ESI representation among New Investigator awards.

FIGURE 13

FIGURE 13: MEDIAN AND MEAN AGES OF NIDDK R01/R37 INVESTIGATORS



Over the past 10 years, the mean and median ages of investigators holding R01/R37 awards (competing and non-competing) increased by approximately 1 year. This observation is consistent with a long-term trend observed across the NIH.

FIGURE 14

FIGURE 14: SUPPORT PIVOTAL CLINICAL STUDIES AND TRIALS—NIDDK HUMAN SUBJECTS RESEARCH FUNDING AS A PROPORTION OF ALL EXTRAMURAL RESEARCH FUNDING

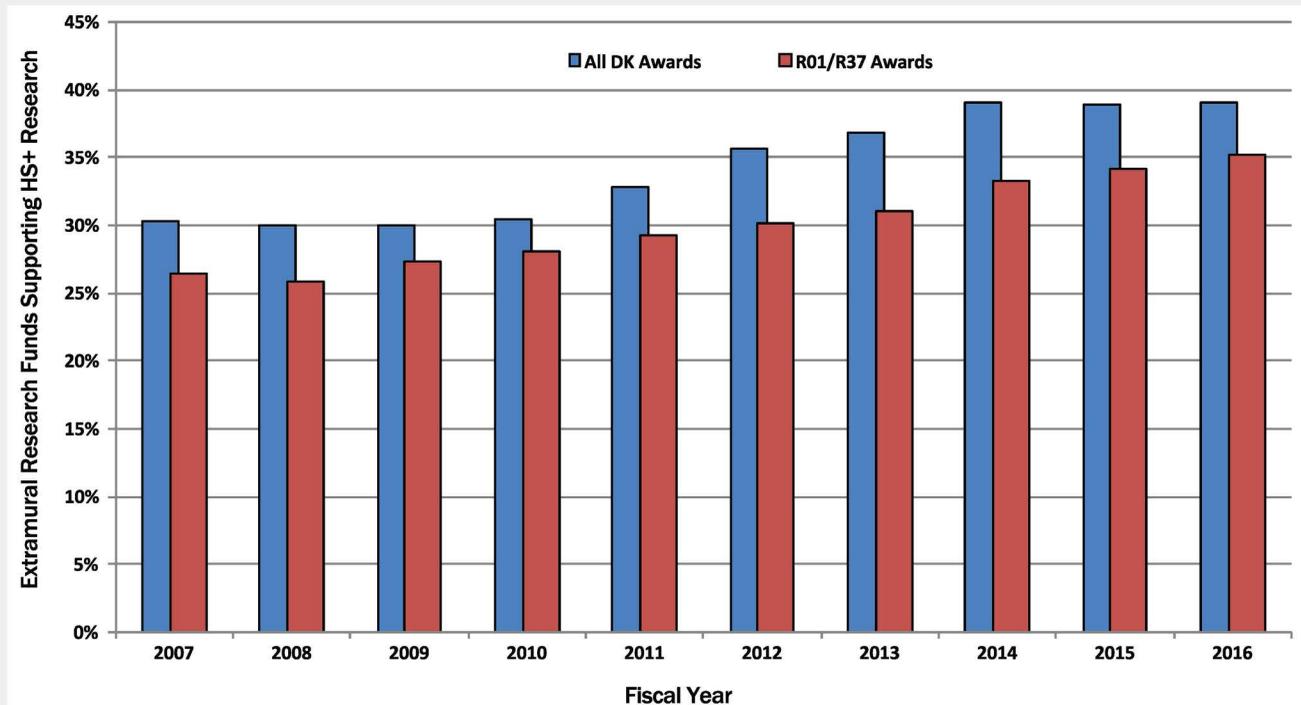


Figure 14 demonstrates that the NIDDK continues to commit a substantial proportion of its research funding to the support of clinical research involving human subjects. For the purpose of this analysis, we used the definition described in Kotchen *et al.*, 2004 (*JAMA* 291: 836-843) and included all studies coded as using human subjects (HS+).

FIGURE 15 A TO 15D

FIGURES 15A TO 15D: THE NIDDK IS COMMITTED TO TRAINING THE NEXT GENERATION OF SCIENTISTS

Figures 15A-D demonstrate that the NIDDK's commitment to training and developing the careers of the next generation of scientists remains strong. Figure 15A shows that overall support of training and career development programs has remained basically stable since FY 2006 and that the slight deceleration of T award support was offset by an increase in support of F awards (by design). Figures 15B and D illustrate that the numbers of NIDDK T awards and associated training slots/positions have remained relatively stable. Figure 15C shows that while the number of NIDDK K08 (Mentored Clinical Scientist Development Awards) has decreased since FY 2007, the numbers of K01 (Mentored Research Scientist Development Awards) and K23 (Mentored Patient-Oriented Research Career Development Awards) have increased. The NIDDK will continue to monitor carefully its training and career development programs to ensure appropriate balance.

FIGURE 15A: NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS AS A PERCENT OF TOTAL EXTRAMURAL RESEARCH FUNDING

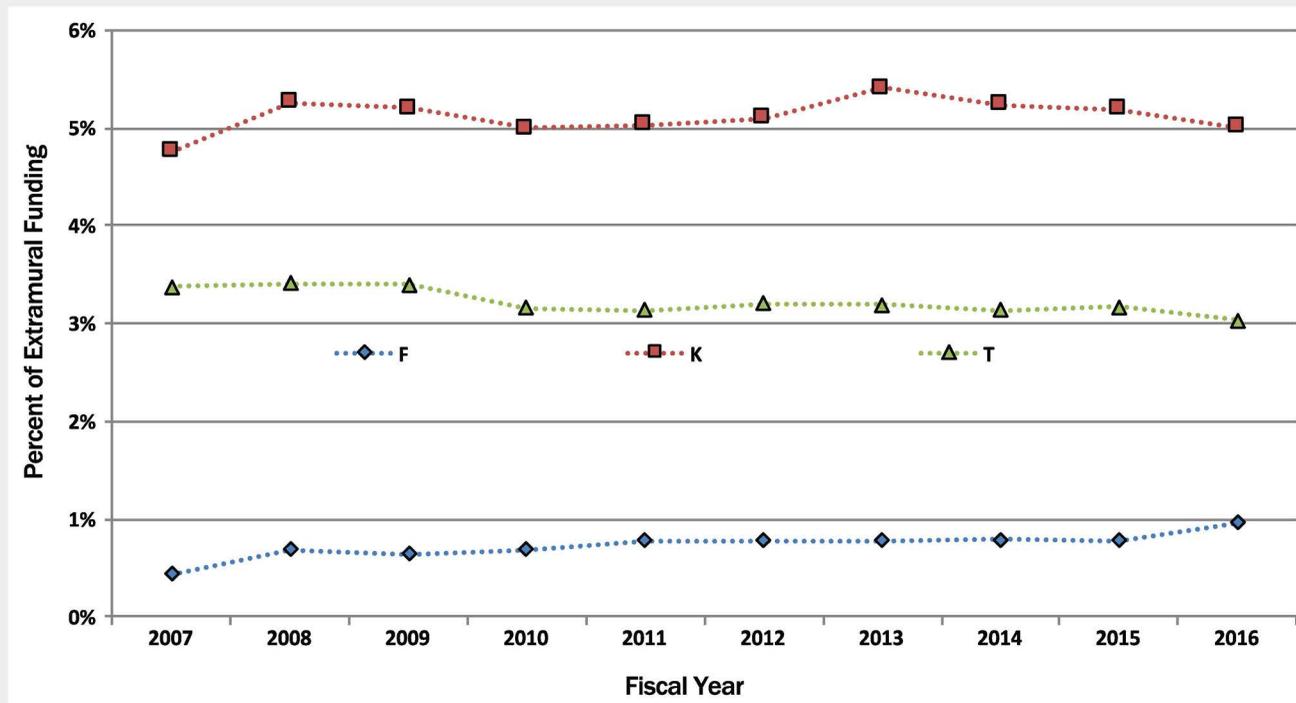


FIGURE 15 A TO 15D

FIGURE 15B: NUMBERS OF NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS BY FISCAL YEAR

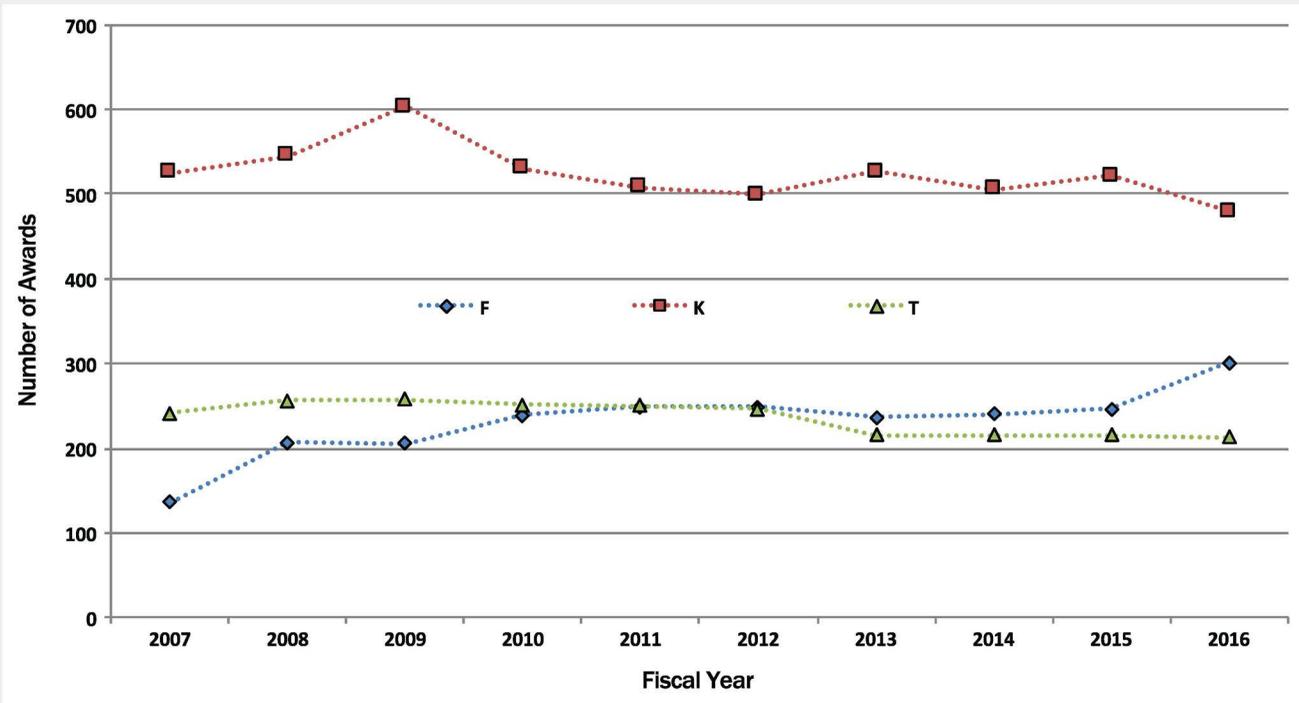


FIGURE 15 A TO 15D

FIGURE 15C: NUMBER OF NIDDK CAREER DEVELOPMENT (K) AWARDS BY ACTIVITY AND FISCAL YEAR

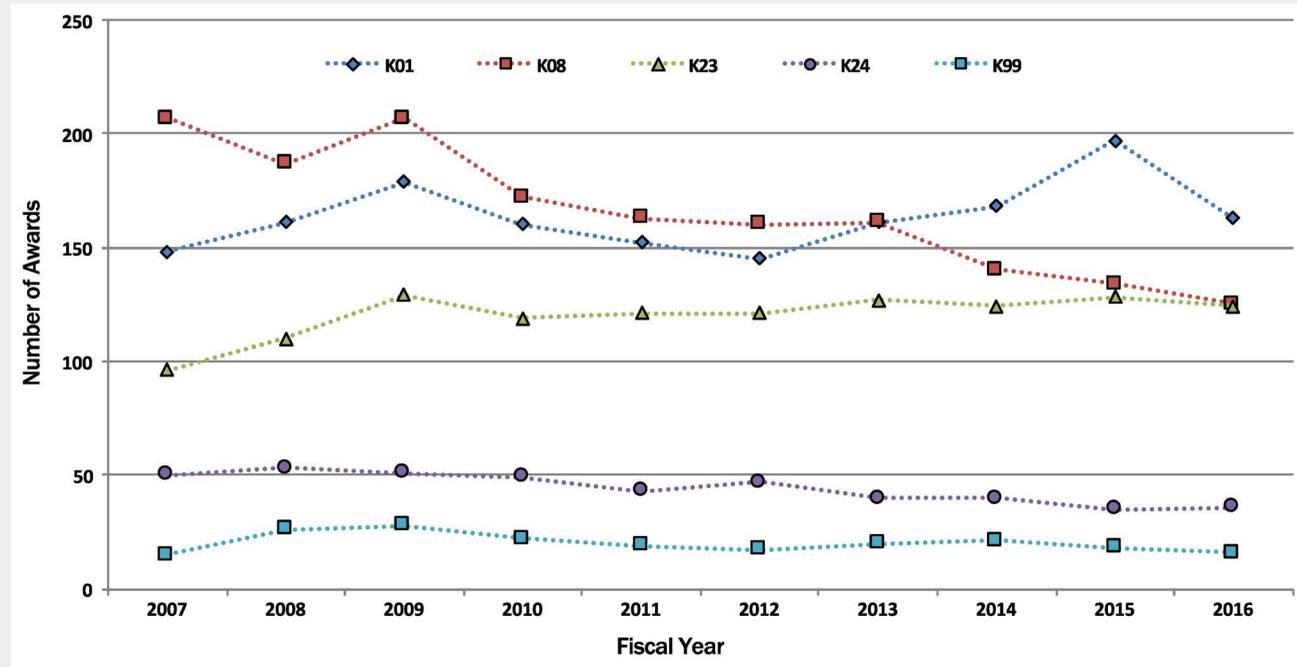
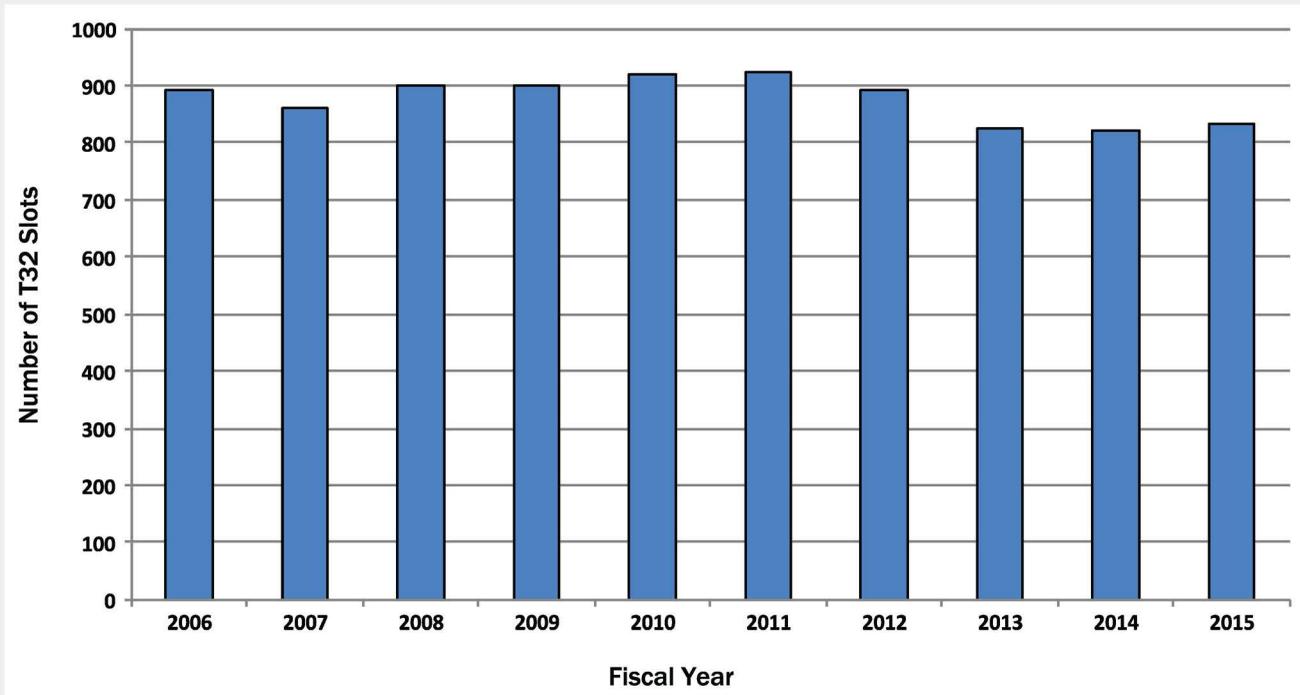


FIGURE 15 A TO 15D

FIGURE 15D: NUMBER OF NIDDK TRAINING (T32) AWARD SLOTS BY FISCAL YEAR



Note: T32 awards made in FY 2016 continue into FY 2017. The total number of T32 slots are reported at the end of the award period. Therefore, the FY 2016 information on T32 slots will not be available until later in FY 2017; thus, unlike the previous charts, FY 2016 data are not included here.

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