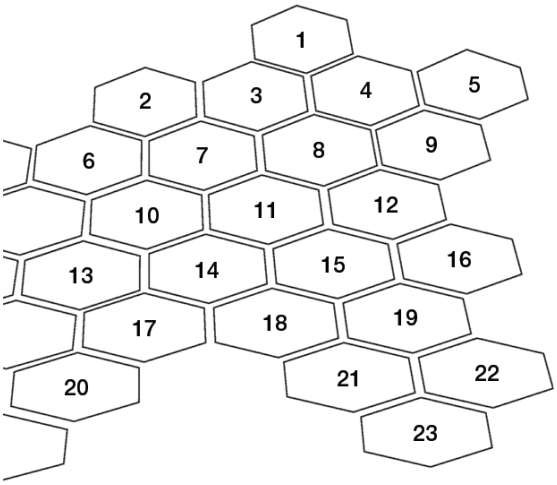
NIDDK

Recent Advances & Emerging Opportunities

February 2011

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

The NIDDK supports basic, clinical, and translational research to understand how networks of biological interactions contribute to normal health and disease. Advances highlighted in this compendium include molecular aspects of protein structure, spatial organization of the human genome, control of gene activity in the development and function of tissue and organ structure, and regulation of interactions with the microbial community in metabolism. While biology involves networks of interactions on different scales, biomedical research often benefits from collaborations and interactions among investigators. Many of the science advances described in this book are the result of NIDDK's broad portfolio of investigator-initiated research, which often involves interactions among the researchers within and between individual laboratories. In addition, NIDDK supports initiatives that promote collaborations among networks of basic, clinical, and translational investigators. Logos for a few of the many research networks supported by NIDDK are shown on the cover and include some of the networks whose research is featured in this compendium. Science advances from investigator-initiated research and basic and clinical research networks will continue to uncover the molecular underpinnings of disease and improve our ability to diagnose, treat, and prevent diseases.



(1) ***CKiD*** *is a prospective cohort study evaluating the risk factors and impact of chronic kidney disease in children.* *(2)* *The* ***Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network*** *brings together clinical, epidemiological, and basic researchers to better understand the causes of chronic urologic pain syndromes.* *(3)* *The goal of the* ***Hepatitis B Research Network*** *is to conduct research on chronic hepatitis B in order to better understand the physiological effects of the disease and to develop effective treatment strategies.* *(4)* *Investigators in* ***The Environmental Determinants of Diabetes in the Young (TEDDY)*** *study are coordinating efforts to identify infectious agents, dietary factors, or other environmental factors that trigger type 1 diabetes in genetically susceptible people.* *(5)* *Four-year-old* ***Nilia Olsen*** *is participating in the TEDDY study to identify what triggers type 1 diabetes in children.* *(6)* ***The Nuclear Receptor Signaling Atlas*** *is a trans-NIH consortium designed to further key areas of research and education in the nuclear receptor and coregulation signaling scientific community.* *(7)* ***The family of Lindsey Duquette*** *shares their experience participating in a NIDDK study testing a new treatment for nephrotic syndrome.* *(8)* ***Type 1 Diabetes TrialNet*** *is an international network of investigators seeking to prevent type 1 diabetes in high-risk people and to preserve insulin function in those newly diagnosed.* *(9)* ***Action for Health in Diabetes (Look AHEAD)*** *is a randomized clinical trial investigating the long-term health benefits of weight loss in overweight and obese people with type 2 diabetes.* *(10)* *The* ***Nephrotic Syndrome Study Network (NEPTUNE)*** *is an integrated group of medical centers, patient support organizations, and clinical researchers dedicated to advancing the understanding and treatment of the most common forms of nephrotic syndrome.* *(11)* *Investigators from the NIDDK* ***Inflammatory Bowel Disease (IBD) Genetics Consortium*** *are conducting studies to identify genes that increase risk for developing IBD.* *(12)* *The* ***National Kidney Disease Education Program (NKDEP)*** *aims to increase awareness of kidney disease and its risk factors, the importance of testing those at risk, and the availability of treatment to prevent or slow the progression to kidney failure.* *(13)* *The* ***Longitudinal Assessment of Bariatric Surgery (LABS)*** *consortium is coordinating clinical, epidemiological, and behavioral research to better understand bariatric surgery and its impact on the health and well-being of people with extreme obesity.* *(14)* *The* ***National Diabetes Education Program (NDEP)*** *is the leading federal government public education program that promotes diabetes prevention and control. The mission of NDEP is to reduce the morbidity and mortality associated with diabetes and its complications.* *(15)* *The* ***Childhood Liver Disease Research and Education Network (ChiLDREN)*** *is a collaborative team of doctors and scientists studying the causes, natural history, and treatment of pediatric liver diseases.* *(16)* *The* ***Diabetes Prevention Program Outcomes Study (DPPOS)*** *is studying the long-term effect of diet and exercise and the diabetes medication, metformin, on the delay of type 2 diabetes in participants of the landmark Diabetes Prevention Program.* *(17)* ***Joan Pasquesi*** *shares her story about participating in the* ***Hyperglycemia and Adverse Pregnancy Outcomes (HAPO)*** *study.* *(18)* *The objectives of the* ***Chronic Renal Insufficiency Cohort (CRIC) Study*** *are to improve understanding of the relationship between chronic kidney disease and cardiovascular disease and to examine risk factors for their progression in people with reduced kidney function.* *(19)* *The* ***Beta Cell Biology Consortium (BCBC)*** *facilitates interdisciplinary research that will advance our understanding of pancreatic islet cell development, regenerative capacity, and function.* *(20)* ***Urinary Incontinence Treatment Network (UITN)*** *investigators conduct clinical studies and trials of the most commonly used surgical, pharmacological, and behavioral approaches for management of urinary incontinence in women.* *(21)* ***David Warren****, a 62-year-old retiree of the U.S. Postal Service, participated in the NIDDK-funded PIVENS trial testing treatments for nonalcoholic steatohepatitis in adults.* *(22)* *Researchers from the* ***Nonalcoholic Steatohepatitis (NASH) Clinical Research Network*** *study the nature and underlying causes of NASH and conduct clinical studies on the prevention and treatment of NASH in adults and in children.* *(23)* *The* ***NIDDK Consortium Interconnectivity Network (dkCOIN)*** *serves the needs of basic and clinical investigators by connecting networks of investigators and providing seamless access to large pools of data relevant to the mission of NIDDK.*

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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 compendium highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility, which includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases, such as cystic fibrosis; liver disease and other digestive diseases, such as inflammatory bowel diseases; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases, such as interstitial cystitis/painful bladder syndrome; and hematologic diseases, such as Cooley's anemia.

The 11th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- Discovery that glucagon-producing alpha cells in the mouse pancreas can convert to insulin-producing beta cells
- Finding that a generic anti-inflammatory drug improves blood glucose control in people with type 2 diabetes
- Revelation of a molecular mechanism that regulates fat cell development and insulin sensitivity
- Demonstration that potential treatments improve nonalcoholic steatohepatitis—a severe form of nonalcoholic fatty liver disease—in adults
- Discovery of genetic variants that increase risk for developing inflammatory bowel diseases in children
- Identification of a drug that may be useful for treating liver disease associated with alpha-1 antitrypsin deficiency
- Demonstration that two operations for treating stress urinary incontinence have similar benefits in helping women achieve improved bladder control
- Discovery that induced pluripotent stem cells retain a molecular memory of the types of cells they were in their pre-pluripotent state
- Identification of a genetic variant that significantly increases the risk of developing kidney disease in African Americans

We also include personal stories of patients. A family dedicated to advancing research on type 1 diabetes describes their participation in a clinical study to determine the environmental factors that trigger the development of type 1 diabetes in children. A nurse shares her story about why she participated in an observational study evaluating maternal blood glucose levels and pregnancy outcomes. A man describes his experience participating in a clinical trial testing new treatments for a form of nonalcoholic fatty liver disease. Parents describe their daughter's battle with nephrotic syndrome and their experience in a clinical study testing a potential treatment for this kidney disease.

The NIDDK is continuing efforts to ensure that knowledge gained from its research advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's major education programs, the National Diabetes Education Program and the National Kidney Disease Education Program. Additionally, the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive

Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available in printed copy and on the NIDDK Web site so that they are readily accessible to patients, health care providers, and the public. I invite you to visit the Web site at: *www.niddk.nih.gov*

We can only reflect a fraction of the immense body of research performed by basic scientists, clinical investigators, and patient volunteers here. We remain committed to translating their efforts 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

The core mission of NIDDK reflects the Director's following 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

60 years 1950 2010
NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

NIDDK: 60 Years of Discovery, 1950-2010

The NIDDK was established as the National Institute of Arthritis and Metabolic Diseases in 1950 by U.S. President Harry S. Truman. Sixty years and four name changes later, one thing has never changed: the Institute's commitment to improving health through high-quality research. In 2010, NIDDK celebrated the rich history of advances made possible through the federal investment in research in our laboratories here in Bethesda, MD, and in Phoenix, AZ, and by grantees around the U.S. and beyond.

NIDDK'S SCIENTIFIC SYMPOSIUM: CELEBRATES PAST, LOOKS TO FUTURE

On Tuesday, September 21, 2010, NIDDK hosted, "Unlocking the Secrets of Science: Building the Foundation for Future Advances," a scientific symposium to highlight research advances made possible, in part, with NIDDK support. NIDDK director, Dr. Griffin Rodgers, welcomed all in attendance to what promised to be an exciting day of cutting-edge scientific presentations. Former NIDDK directors Dr. Lester B. Salans (Mount Sinai Medical School and Forest Laboratories), Dr. Phillip Gorden (NIDDK), and Dr. Allen M. Spiegel (Albert Einstein College of Medicine) chaired scientific sessions on Diabetes and Digestive and Kidney Diseases; Nutrition, Hematology, and Urology; and Endocrinology, Education, Outreach, and the NIDDK Intramural Research Program. Each session featured three presentations from distinguished scientists spanning NIDDK's research mission.

Photo: Ernie Branson

Session 1: Diabetes and Digestive and Kidney Diseases C. Ronald Kahn, M.D., ***Insulin Resistance and Metabolic Disease: An Integrative Systems Biology Approach*** Jeffrey Gordon, M.D., ***Our Human Gut Microbiome: Dining in with Trillions of Fascinating Friends*** Eric Neilson, M.D., ***The Origin of Fibroblasts During Tissue Fibrogenesis***
Session 2: Nutrition, Hematology, and Urology Jeffrey Friedman, M.D., ***The New Biology of Obesity*** Nancy Andrews, M.D., ***New Roles for the Classical Transferrin Receptor*** John McConnell, M.D., ***Benign Prostatic Hyperplasia: It's Not as Simple as We Thought***
Session 3: Endocrinology, Education, Outreach, and Intramural Jeffrey Flier, M.D., ***Resistance to Action: Hormonal and Decanal*** James R. Gavin, III, M.D., ***Transforming Science into Educational Programs: NIDDK and 60 Years of Outreach*** Gary Felsenfeld, Ph.D., ***Chromatin Boundaries, Insulators, and the Epigenetic Regulation of Gene Expression***

One way the Institute is building on its accomplishments is by nurturing early career scientists, 12 of whom were recognized and selected to receive the NIDDK 60th Anniversary Early Career Investigator/Scholar Awards for 2010. By demonstrating leadership, creativity, and excellence in their pursuit of scientific knowledge, they are building on the foundation of NIDDK research in the past and achieving the progress of medical research in the future. Working on the NIH campus and at grantee institutions throughout the U.S., these men and women represent the promise of a future that will continue to benefit the health and longevity of generations of people throughout our Nation and the world.

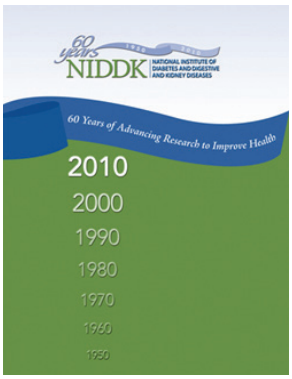
One clear message took shape as the day-long symposium came to a close, research has laid the groundwork for today's discoveries, and NIDDK looks forward to building on this progress to propel scientific discovery in the years ahead toward improving the Nation's health.

Three former directors of NIDDK joined current NIDDK Director Dr. Griffin Rodgers (second from left) at the Institute's 60th anniversary symposium. At left, former directors Dr. Lester B. Salans; second from right, Dr. Phillip Gorden; at right, Dr. Allen M. Spiegel.
Photo: Ernie Branson

During the poster session, Dr. Daniel Appella (NIDDK) discusses his research findings with NIDDK Deputy Director Dr. Gregory Germino.
Photo: Ernie Branson

NIDDK 60th Anniversary Early Career Investigator/Scholar Awards. Seated left to right: Ajay Chawla, M.D., Ph.D., Stanford School of Medicine; Martin T. Zanni, Ph.D., University of Wisconsin; Clara Abraham, M.D., Yale University; Karen Guillemin, Ph.D., University of Oregon; Matthias H. Tschöp, M.D., University of Cincinnati; Laura M. Calvi, M.D., University of Rochester Medical Center. Standing left to right: Jeremy S. Duffield, M.D., Ph.D., University of Washington, Seattle; Daniel Appella, Ph.D., NIDDK; Susan Buchanan, Ph.D., NIDDK; Orna Cohen-Fix, Ph.D., NIDDK; Alexandra C. McPherron, Ph.D., NIDDK; Kristin V. Tarbell, Ph.D., NIDDK. Standing right: Dr. Griffin Rodgers presented the awards. Photo: Ernie Branson

NIDDK: 60 YEARS OF ADVANCING RESEARCH TO IMPROVE HEALTH



The publication *NIDDK: 60 Years of Advancing Research to Improve Health* celebrates the Institute's accomplishments over the past 60 years in supporting and conducting research on some of the most common, chronic, and costly diseases affecting people in this country and around the world, as well as on diseases and disorders that are less widespread but nonetheless devastating in their impacts. Additional information on this compendium can be found here: *www2.niddk.nih.gov/AboutNIDDK/ReportsAndStrategicPlanning/SixtiethAnniversary/*

CONGRESSIONAL BREAKFAST

Members of Congress, congressional staffers, and representatives of many scientific and patient advocacy organizations celebrated the 60th anniversary with a congressional breakfast on Capitol Hill. The coalition presented NIDDK Distinguished Scientist Awards to two NIDDK grantees and a former NIDDK director. Dr. David Nathan (Massachusetts General Hospital) was recognized for his leadership and vision in NIDDK-supported diabetes research. Dr. Jeffrey Gordon (Washington University in St. Louis) was cited for his groundbreaking work on the role of bacteria residing in the human digestive tract in health and disease. Dr. Phillip Gorden, a former NIDDK director, was honored for both his leadership as NIDDK director and for his research on insulin action and insulin resistance in diabetes. In addition, the current NIDDK director, Dr. Griffin Rodgers, presented the NIDDK Early Career Investigator Award to Dr. Theo Heller (NIDDK Liver Diseases Branch) for his research on basic and clinical aspects of viral hepatitis.

Dr. Griffin Rodgers (*l*) chats with (*from l*) Ms. Kim Hollander and Mr. Brett Rosen of the Oxalosis and Hyperoxaluria Foundation and U.S. House Representative Nita Lowey (D-NY).
Photo: Bill Branson

From left: Drs. David Nathan, Jeffrey Gordon, Theo Heller, and Phillip Gorden
Photo: Bill Branson

111th Congress
2nd Session

H. RES. 1444

Recognizing the 60th anniversary of the National Institute of Diabetes and Digestive and Kidney Diseases

IN THE HOUSE OF REPRESENTATIVES
JUNE 15, 2010

Mr. PALLONE (for himself and Mr. SHIMKUS) submitted the following resolution; which was referred to the Committee on Energy and Commerce

RESOLUTION

Whereas the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) leads the Nation's Federal commitment to research, research training, and science-based education and health information dissemination with respect to diabetes and other endocrine and metabolic diseases, digestive and liver diseases, nutritional disorders, obesity, kidney diseases, urologic diseases, and hematologic diseases;

Whereas the Institute was originally established in 1950 through the Omnibus Medical Research Act as the National Institute of Arthritis and Metabolic Diseases, was renamed several times during the ensuing decades, and was renamed in 1986 as the National Institute of Diabetes and Digestive and Kidney Diseases;

Whereas the chronic and costly diseases and disorders within the Institute's mission together affect millions of Americans, and range from some of the Nation's most common diseases and disorders to those which are more rare;

Whereas the Institute supports research by extramural scientists at academic and other medical research institutions across the Nation, as well as research by scientists in the Institute's intramural program;

Whereas the Institute supports basic, clinical, population, and translational research in laboratory, clinic, and community settings throughout the country;

Whereas the NIDDK pursues research efforts to benefit all individuals burdened by diseases and disorders within the Institute's mission, including both men and women, older and younger adults, children, minority populations who are disproportionately affected by many of these diseases, and people from economically disadvantaged backgrounds;

Whereas NIDDK-supported research discoveries have dramatically increased vital understanding of the biologic mechanisms and behavioral and environmental factors that contribute to health and disease—knowledge which has propelled the development of intervention strategies;

Whereas research conducted and supported by the NIDDK has been instrumental in revolutionizing prevention, diagnosis, and treatment strategies for individuals who have, or are at risk for, diseases and disorders within the Institute's mission, leading to remarkable improvements in health and quality of life;

Whereas the NIDDK has been a leader in research training and mentoring efforts, from summer programs for high school and college students with special opportunities for underrepresented minorities, to fellowships for graduate and medical students and postdoctoral researchers, to support for early-career and established investigators, in order to ensure that critical biomedical research will continue into the future;

Whereas the Institute additionally sponsors education and outreach programs, with materials tailored for diverse audiences, to improve health by disseminating science-based information to patients and their families, those at risk for disease, health care professionals, and the general public;

Whereas the Institute has been a leader in collaborative and coordinated research efforts and science-based education programs, in order to maximize the Federal investment in research and synergize expertise across the National Institutes of Health, with other Federal agencies, and with public and private organizations;

Whereas the burden of diabetes, endocrine and metabolic diseases, digestive diseases, nutritional disorders, obesity, and kidney, urologic, and hematologic diseases remains a public health challenge for the Nation; and

Whereas NIDDK-supported investigators continue to make strides in research toward understanding, preventing, and treating type 1 diabetes; type 2 diabetes; gestational, monogenic, and other forms of diabetes, and diabetic complications; other endocrine and metabolic diseases, including cystic fibrosis, osteoporosis, and lysosomal storage disorders; digestive diseases, including those affecting the gastrointestinal tract, pancreas, liver, and biliary system; inflammatory bowel disease; nutritional disorders, including Celiac disease; obesity; kidney diseases, including chronic kidney disease and acute kidney injury, polycystic kidney disease, focal segmental glomerulosclerosis, end-stage renal disease, and other kidney diseases; urologic diseases and disorders, such as urinary incontinence, urinary tract infections, interstitial cystitis/painful bladder syndrome, chronic prostatitis/chronic pelvic pain syndrome, and kidney stones; and hematologic diseases, including anemias and other blood disorders: Now, therefore, be it

Resolved, That the House of Representatives—
(1) commemorates the 60th anniversary of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and commends the Institute for its leadership in research, research training, and science-based education programs; (2) recognizes—(A) the Institute-supported extramural and intramural scientists whose studies have improved human health, and whose research continues to yield promising discoveries; (B) the volunteers who participate in clinical studies; and (C) the patient and professional health organizations who contribute to the shared research goals of preventing, treating, and curing the diseases and disorders within the Institute's mission; and (3) reaffirms support for the NIDDK and its continued commitment to research to improve health.

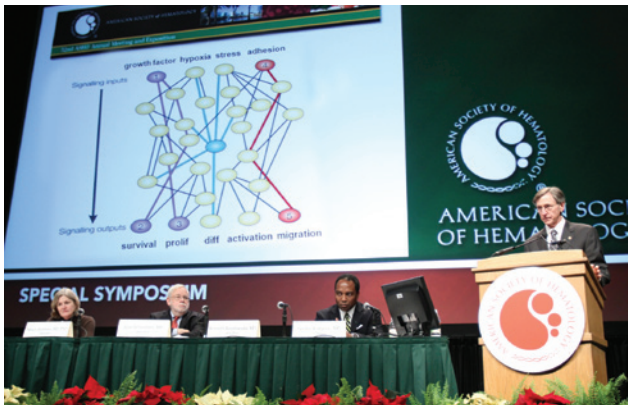
EVENTS HELD IN 2010

Several professional research societies partnered with NIDDK to celebrate the 60th anniversary by holding special events during their annual meetings.

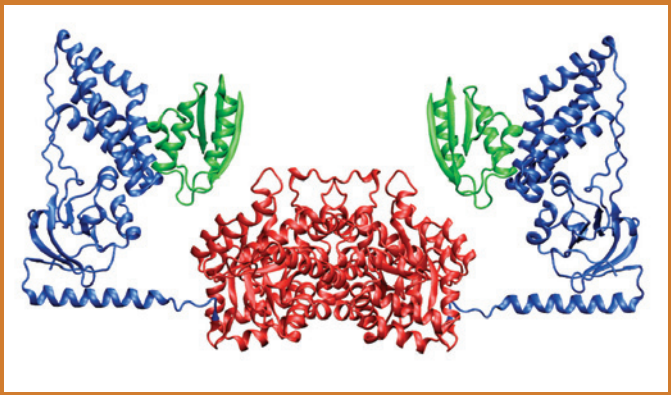
May 1	American Society of Pediatric Nephrology
May 2	American Gastroenterological Association
May 31	American Urological Association
June 19-22	The Endocrine Society
June 26	American Diabetes Association
July 9-10	International Society on Hypertension in Blacks
July 14	Society for the Study of Ingestive Behavior
Aug. 2	National Medical Association
Aug. 7	Association of American Indian Physicians
Aug. 22	American Chemical Society
Sept. 21	NIDDK's Research Symposia
Oct. 8 & 10	The Obesity Society
Oct. 15-19	American Society for Bone and Mineral Research
Oct. 29-Nov. 2	American Association for the Study of Liver Diseases
Nov. 2-6	American Society of Human Genetics
Nov. 16-21	American Society of Nephrology
Dec. 4-7	American Society of Hematology

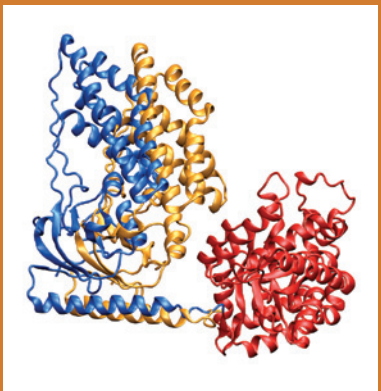
An NIDDK symposium on diabetes and obesity, held on June 26 at the American Diabetes Association Scientific Sessions in Orlando, FL.
Photo: Lagniappe Photography, courtesy of the American Diabetes Association

From left: Dr. Charles N. Rotimi, National Human Genome Research Institute; Dr. Suzanne Nicholas, University of California Los Angeles; Dr. Lawrence Agodoa, NIDDK; Dr. Griffin Rodgers, NIDDK; and Dr. Shawna Nesbitt, president of the International Society on Hypertension in Blacks (ISHIB); at the July 9-10 annual ISHIB meeting in Arlington, VA.
Photo: Courtesy of the International Society of Hypertension in Blacks



A special NIDDK 60th anniversary symposium held during the 52nd annual meeting of the American Society of Hematology in Orlando, FL. From left: Dr. Nancy Andrews, Duke University; Dr. Alan Schechter, NIDDK: Dr. Griffin Rodgers, NIDDK; and Dr. Kenneth Kaushansky, Stony Brook University.
Photo: Armando Solares, courtesy of the American Society of Hematology





Research highlighted in this chapter describes a novel method for determining the solution structures of large complexes of proteins in an environment that closely mimics that within a cell. An important part of biomedical research is to understand the relationship between a protein's structure and biological function. Scientists from NIDDK's Intramural Research Program used a combination of biophysical techniques (specifically nuclear magnetic resonance spectroscopy and solution X-ray and neutron scattering) and novel computational approaches to determine how different bacterial proteins fit together to form a large molecular complex that regulates the transport of sugar molecules in and out of the bacterial cell. The image on the left illustrates how the proteins—called Enzyme I (blue/red) and HPr (green)—interact to form a complex, and the image on the right reveals changes in the structure of Enzyme I that occur on forming the complex that may be important for the function of this bacterial enzyme. This advance in methodology provides a new approach for researchers to study the structures of large proteins and protein complexes. Understanding the relationship between a protein's structure and function is an important part of biomedical research, as proteins carry out many of the biological functions underlying normal health and disease.

Graphics provided by Dr. G. Marius Clore, NIDDK.

Cross-Cutting Science

Advances in medicine are largely dependent on the accumulation of new knowledge about biologic processes, often at the smallest levels of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other. Major strides in fighting disease can be traced to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Opportunities to make exciting new discoveries are arising ever more rapidly with the development of new technologies, new approaches, and even new scientific disciplines as teams of talented, creative researchers join together to pursue increasingly complex challenges. Described in this chapter are several recent studies, each of which spans multiple areas within the NIDDK research mission. The insights gained through this research can be expected to aid progress in many scientific endeavors, for today's research advances may lead to tomorrow's cures.

IMPLEMENTING THE AMERICAN RECOVERY AND REINVESTMENT ACT OF 2009 AT NIDDK

On February 17, 2009, President Barack Obama signed into law the American Recovery and Reinvestment Act of 2009 (Recovery Act; Public Law 111-5). With potential for biomedical research to stimulate the economy, NIH received a generous infusion of funding from the Recovery Act, and this funding was distributed throughout NIH.

Poised to capitalize on this unique investment opportunity, NIDDK developed a funding plan to meet the stimulus goals of the Recovery Act and to advance scientific progress. The funding supported a range of biomedical research efforts across the Institute's research mission. As a priority, the Institute remains committed to funding outstanding science as judged by the NIH peer review process. The Recovery Act provided an opportunity for NIDDK to support many highly meritorious, peer-reviewed research project grants that met program priorities and could be realistically accomplished within a 2-year funding period. In addition, NIDDK was able to provide short-term funding for many peer-reviewed applications to help investigators continue ongoing studies, maintain laboratory personnel, and get exciting projects off the ground.

The NIDDK used Recovery Act funds to accelerate and expand research in two "Signature" programs. The "Novel Cell Therapies in Regenerative Medicine for Diabetes" Signature Program is investigating novel human islet cell replacement therapies for patients with type 1 diabetes. Replacement of insulin-producing pancreatic islet beta cells holds great promise for the treatment and cure of type 1 diabetes. The "Genome-Wide Association Studies and Replication in Diseases of Interest to NIDDK" Signature Program is identifying genetic variations associated with diseases within the NIDDK mission. High priority areas for this program included extending studies to minority populations and to diseases that have not been addressed in previous genome-wide studies. Once new genetic associations are identified, researchers can use the information to develop better strategies to detect, treat, and prevent diseases.

In addition to supporting Signature Programs, NIDDK used Recovery Act funds to supplement ongoing projects that span all aspects of its research mission. Administrative Supplements to existing grants provided resources for additional lab personnel and research equipment. Through the Recovery Act, NIDDK also was able to provide additional funding to advance many of its ongoing major research initiatives, such as the Beta Cell Biology Consortium, the Diabetes Prevention Program Outcomes Study, the Halt Progression of

Polycystic Kidney Disease (Halt-PKD) clinical trial, and the Action for Health in Diabetes (Look AHEAD) clinical trial. In addition to supporting existing programs, NIDDK was able to use the Recovery Act to begin a new program—the NIDDK Consortium Interconnectivity Network, or "dkCOIN"—which serves to connect investigators, resources, and data from various NIDDK research networks.

The Recovery Act also provided funds for investment in new high-impact initiatives that will affect the course of future research. The NIDDK participated in the NIH Challenge Grants in Health and Science research initiative. This grant program was created to support research addressing specific challenges in biomedical and behavioral research that would benefit from significant 2-year jumpstart funds. The NIDDK used the Recovery Act to support high-impact, peer-reviewed Challenge Grants in the areas of enabling technologies, stem cells, clinical research, translational research, and comparative effectiveness research.

In addition to stimulating the U.S. economy, NIDDK expects the Recovery Act to stimulate a number of scientific areas that advance its research mission. The return on this investment is already evident, with a number of scientific advances funded, in part, by the Recovery Act highlighted in this research compendium. It is likely that these and other exciting new findings that result from the Recovery Act investment will answer many pressing research questions and open entirely new areas of biomedical research.

RECENT GENETIC STUDIES: PAVING THE WAY TO IMPROVING PEOPLE'S HEALTH

Genetics, the study of genes—the basic unit of inheritance—is one of the tools in a researcher's arsenal to understand human health and disease. Understanding genetic contributions to the development of disease can lead to clues to biological pathways involved that are driven, at least in part, by variations in genes and their regulatory regions, many of which remain to be identified. Some gene variants directly cause disease, while others confer susceptibility to disease in combination with other genes or environmental factors. Not only will genetics research open new avenues for developing therapies and invigorate the drug development pipeline, it also holds promise to advance development of personalized therapy.

In a breakthrough that revolutionized the field of human genetics, scientists realized that variations in DNA sequences, such as those called "single nucleotide polymorphisms," or SNPs, can be used as genetic markers for mapping disease-associated genes. The Human Genome Project provided a comprehensive map of genetic sequences and the Haplotype Map provided a catalog of genetic variation. Additionally, scientists devised tools and methods to more rapidly and routinely analyze DNA sequences and variations. These advances led to genome-wide association (GWA) studies, in which researchers have compared variants across the entire genome from individuals with and without a particular disease so as to pinpoint genetic regions, called loci, that track with the disease and may thus harbor a disease gene.

These new research tools have enabled an explosion of advances in understanding the genetic basis of complex human diseases and have identified hundreds of disease-associated gene loci, uncovering new knowledge of disease mechanisms. For example, unexpected pathways have been implicated in disease, and previously unknown associations between diseases have been illuminated. Findings from GWA investigations and other advanced genetic studies also indicate that common diseases result from the influence of multiple risk genes with varying contributions and interactions with environmental factors. A more complete understanding of genetic variation contributing to disease will be available as comprehensive sequencing methods are undertaken, which will uncover rarer variants.

The NIDDK has supported many advances in genetics research related to diseases and disorders within its mission. For example, as recently as 2005 only two genes were known to affect type 2 diabetes. Today, there are over 40 new gene loci associated with risk of type 2 diabetes, many of which were found by NIDDK-supported studies. Similarly, in 2003 only three genes were known to affect type 1 diabetes. Today, the NIDDK-led Type 1 Diabetes Genetics Consortium (T1DGC) and its collaborators have identified over 40 gene loci associated with risk of type 1 diabetes, bringing the total number of known

regions to nearly 50. NIDDK-supported consortia like the Family Investigation of Nephropathy and Diabetes, the Genetics of Kidneys in Diabetes Study, and the Epidemiology of Diabetes Interventions and Complications study have collected a wealth of genetic data, which has been deposited in the Database for Genotype and Phenotype (dbGAP), and researchers continue to mine this information to elucidate the genetic contributors to diabetes complications. In addition, novel genetic loci associated with healthy blood glucose levels are being identified, providing new insight into the biology of metabolism. See the Diabetes, Endocrinology, and Metabolism (DEM) chapter for more information on this specific advance.

In another example, NIDDK-supported researchers have discovered genetic regions associated with inflammatory bowel diseases (IBD). As reported in the Digestive Diseases and Nutrition (DDN) chapter of this compendium, the NIDDK Inflammatory Bowel Disease Genetics Consortium recently identified several susceptibility loci for ulcerative colitis, a form of IBD that causes inflammation in the tissues lining the colon and rectum. In this advance, approximately 30 gene loci were implicated in ulcerative colitis. The same chapter also describes the identification of five new genetic variations that predispose children to developing IBD. As other genetic risk factors for IBD typically have been identified in adults, this advance extends these studies to pediatric populations.

Researchers have also made strides in determining the genetic factors that influence immune-mediated digestive diseases. As described in the DDN chapter of this report, NIDDK-supported scientists have now identified disease-associated genetic variants within a cluster of genes that play an important role in the immune system. These variants affect multiple chronic inflammatory diseases with autoimmune features, including Crohn's disease and ulcerative colitis, the two major forms of IBD. New genetic risk factors have also been uncovered in celiac disease, an autoimmune disease in which an aberrant immune response to the gluten protein found in many dietary grains results in chronic inflammation and tissue damage in the intestine. This research is also described in the DDN chapter.

Kidney disease research has also gained new insights from advanced genetics studies. In one study described in the Kidney, Urology, and Hematology (KUH) chapter of this report, NIDDK-supported researchers identified 13 new genetic loci affecting renal function and chronic kidney disease, and seven loci suspected to affect production and secretion of a protein found in urine. Another group of NIDDK-supported researchers reported that variants around the *MYH9* gene are linked to susceptibility to various forms of kidney disease among African Americans. Further research, also described in the KUH chapter, revealed that much of the kidney disease risk is actually due to variants in an adjacent gene, *APOL1*. These variants likely protect against a trypanosomal infection that causes African sleeping sickness, a degenerative and potentially fatal disease affecting tens of thousands of people in sub-Saharan Africa. This finding may lead to the development of better treatments for both chronic kidney disease and African sleeping sickness.

Challenges remain, however, in translating these exciting genetics findings to the development of new therapeutics and improvements in health. First, many disease-associated variants have been identified in populations of European origin. To ensure that all Americans benefit from the fruits of these studies, it will be necessary to conduct similar studies in ethnically and racially diverse populations. The NIDDK is promoting such research; for example, a new initiative, the Type 2 Diabetes Genetic Exploration by Next-generation sequencing in multiEthnic Samples (T2D-GENES) Consortium, is investigating and comparing the genetic causes of diabetes in multiple ethnic groups. Second, while scientists have identified an impressive number of loci associated with various diseases, for a number of these loci the causal gene—the gene and precise mutation that influence disease risk—is not yet known. This Consortium will be sequencing within the loci, in multiple populations, to see if different mutations contribute to diabetes in different populations. Third, once the exact gene associated with disease risk has been identified, research is required to determine how and to what extent the gene affects disease risk or disease progression.

Another critical challenge is to bridge the gap from understanding disease development and progression to efficacious prevention and treatment strategies. Prediction of disease risk—based on an individual's genome—will be useful if proven interventions for

specific patient populations exist. Genetic prediction of disease risk, however, may also help advance the development of interventions. For example, scientists can identify, in part from genetics, individuals at heightened risk for type 1 diabetes; this knowledge gives people the opportunity to volunteer for clinical trials to test potential prevention strategies. As part of the NIH Genes, Environment, and Health Initiative, NIDDK leads two initiatives to bring genetic knowledge to clinical utility. One initiative focuses on the translation of significant genetic findings into clinical or public health use. The other initiative supports research to measure the responses of patients and providers to information about genetic determinants of common diseases and to determine how to effectively educate the public to use the information appropriately for clinical care and disease prevention.

Genetic studies are an important component of the research to improve understanding of disease and to develop better predictions of risk, new prevention strategies, and novel treatment approaches. In addition, studies in different ethnic populations may uncover a genetic contribution to health disparities. With continued research, NIDDK can capitalize on exciting genetic findings to improve the health of Americans at risk for and burdened by these diseases and disorders.

THE MOLECULAR LIBRARIES PROGRAM: DRUG DISCOVERY AT THE INTERSECTION OF BIOLOGY, CHEMISTRY, AND TECHNOLOGY

Recent biomedical research, in particular sequencing of the human genome, has produced enormous potential for the identification of novel proteins and biological pathways that could serve as molecular targets for new drugs. To understand the normal functions of these new targets and determine their roles in disease, scientists require molecular tools for research. "Small molecule" probes—a class of chemical compounds that can interact with and help define the functions of biological molecules—are widely used as experimental research tools and for therapeutic drug development. Great effort is needed to identify a suitable small molecule for a given target. Specially-developed experimental tests (known as assays) determine a small molecule's ability to modulate the activity of the target protein or pathway.

To identify just one or a few promising small molecule probes, hundreds of thousands of compounds may be tested using these assays, and thus high-throughput screening instrumentation is often required. The chemical structures of candidate small molecules are then fine-tuned through "medicinal chemistry" to make their activity as potent and specific as possible.

For many years, the pharmaceutical and biotechnology industries have utilized high-throughput screening technologies, but similar resources had been lacking for researchers in the public sector, who are addressing different biomedical questions. In addition, while some academic biomedical scientists include small molecule discovery in their research, most have never engaged in this challenging process and thus lack the requisite specialized knowledge and experience. To bridge the gap between the biologists in academia and scientists with expertise in small molecule development and high-throughput screening, the Molecular Libraries Program (MLP) was launched as an initiative of the NIH Roadmap.

The MLP is a trans-NIH effort that established nine small molecule production and screening facilities located in California, New Mexico, Kansas, Tennessee, Alabama, Maryland, Florida, and Massachusetts. These centers, collectively called the Molecular Libraries Screening Centers Network, work as a consortium to provide a "library" of chemical compounds, drug discovery expertise, and automated high-throughput capabilities to identify previously unavailable probes that researchers can use to study their target proteins of interest.

Identifying a functional small molecule is no easy task; to be useful for research, numerous strict criteria must be met. For example, the compound must effectively modulate the activity of the desired protein or pathway but should also be specific, *i.e.*, it should interact only with its intended target to avoid unwanted side effects. The ability to screen vast numbers of compounds improves the likelihood of successfully identifying a small molecule capable of fulfilling the necessary rigorous requirements.

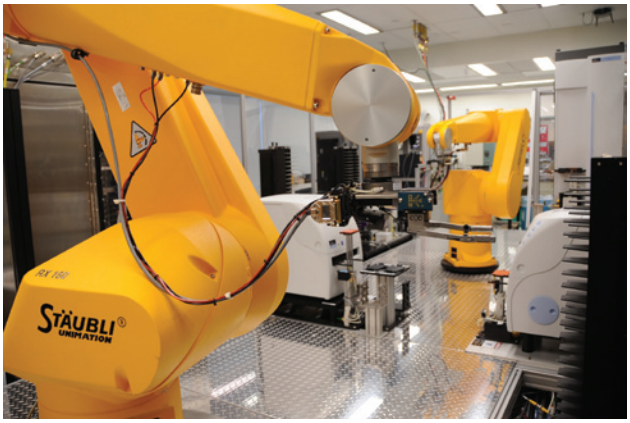
Once discovered, new small molecule probes are added to a growing collection of chemical compounds stored in the Molecular Libraries Small Molecule Repository. Related information (about the chemicals

and assays) is catalogued in a public internet database called PubChem (*http://pubchem.ncbi.nlm.nih.gov/*), which is widely used by biomedical scientists, and to date includes information for more than five million chemical compounds. With vast information from previous research, the PubChem directory provides a valuable starting point for new efforts to identify small molecules that may have useful effects on a broad range of biological processes or diseases.

The NIH Chemical Genomics Center (NCGC), located within the National Human Genome Research Institute, is one of the nine Centers and illustrates the Centers' capabilities. In this comprehensive facility, 75 scientists use state-of-the-art automation to address most aspects of small molecule discovery, including assay design, high-throughput screening, and medicinal chemistry. One major interest of the NCGC is in rare or neglected diseases, which are important areas of academic research and are less likely to be addressed by the private sector. Many projects have been completed by this facility. As just one example, the NCGC developed chemical probes to better understand a rare genetic disorder known as Gaucher's disease. People with this disease have mutations in a protein called glucocerebrosidase. These mutations change the molecule's three-dimensional shape and alter its function, leading to symptoms that include spleen and liver enlargement, neurological disorders, and osteoporosis. The NCGC developed chemicals that interact with this protein and correct its structure. These probes are now being used in the laboratory to better understand the protein's function and are promising candidates for future drug development for possible use in humans.

Chemical probes offer great potential to accelerate the progress of biomedical research. Independently of the MLP, an increasing number of academic scientists are conducting small molecule discovery research in their own laboratories. The MLP complements those efforts by greatly expanding the reach of chemical libraries and high-throughput screening technology to many other biomedical researchers. The chemical probes generated by scientists in the MLP, partnered with academic researchers, have served as useful tools to address challenging research problems, and could potentially lead to new therapies for people suffering from disease.

For more information, please visit: *http://mli.nih.gov/mli*



Automation is a key component of high-throughput screening. Using specially developed assays, robots and machines rapidly screen small molecule libraries, which can contain hundreds of thousands of chemical compounds. A series of steps comprises each assay utilizing different machines—ultimately leading to the identification of small molecules that have a desired function. The particular steps vary depending on the experimental procedure. In this picture, two robots (yellow machines in the foreground and background) operate at different stages of the assay process to transfer individual plates containing large numbers of independent miniature-scale tests stepwise from one automated station to another, until the assay is complete and the results can be analyzed. The machines at these stations can work around the clock to perform these repetitive experimental steps, reducing the time it takes to test the vast collection of small molecule compounds.

STEM CELLS

Induced Pluripotent Stem Cells Find It Difficult To Forget: Researchers have recently discovered why induced pluripotent stem (iPS) cells don't always function as well as embryonic stem (ES) cells. Human ES cells are "pluripotent"—that is, they have the ability to form virtually any cell type in the body and may thus possess the ability to repair human tissues and organs. They also can be propagated indefinitely in the laboratory. However, the use of ES cells is controversial because their isolation entails the destruction of early-stage human embryos.

In recent years, scientists have developed two different ways of reprogramming adult cells, such as those derived from blood or skin, to revert back to an ES cell-like state, with the potential to give rise not only to new cells of their original type but also to more

stem cells and a multitude of different types of cells. One technique involves adding just four genes that convert adult cells into iPS cells. The other technique entails transferring the nucleus from an adult cell into a fertilized egg from which the original nucleus has been removed. The egg then reprograms the adult nucleus, enabling the isolation of pluripotent stem cells with the genetic makeup of the adult cell. Both types of cells could be used to model diseases and potentially to create cells to treat specific diseases, but the use of human pluripotent stem cells generated by nuclear transfer has ethical issues similar to those of traditional ES cells.

The ability of iPS cells to form cells of lineages other than the type from which they were originally derived has been limited, and scientists sought to determine why this was the case. In this study, conducted with mouse cells, the research team examined the role played by DNA methylation. DNA methylation is a type of modification of the genetic material where small chemicals, called methyl groups, are attached to various parts of DNA. Although this process does not alter the sequence of the genetic code, DNA methylation can affect the cell's ability to activate or deactivate genes. Different types of cells have different characteristic methylation patterns. The scientists found that mouse iPS cells retain residual DNA methylation reflecting their tissue of origin. The methylation patterns serve as a kind of "memory," affecting gene expression and restricting the number and kind of ultimate fates of the cells. Moreover, the researchers discovered that compared to iPS cells, nuclear transfer-derived pluripotent cells were more similar to ES cells in their methylation patterns and their ability to form a wider variety of different cell types. The researchers did find that the methylation memories of iPS cells could be reset, at least in part, either by growing the cells longer in the laboratory or by treating them with drugs that affect DNA methylation.

Cautious optimism continues to describe the eventual use of iPS cells for experimental models of disease, as targets in drug screening studies, and as a source for regenerating tissue.

Kim K, Doi A, Wen B, et al. Epigenetic memory in induced pluripotent stem cells. Nature 467: 285-290, 2010.

Reprinted, in slightly modified form, from NIH Research Matters*; original article by Harrison Wein, Ph.D., published on August 2, 2010.*

INSIGHTS INTO PROTEIN STRUCTURE

Novel Approach for Determining the Structure of Large Protein Complexes: Scientists from NIDDK's Intramural Research Program have developed a novel approach for determining the structure of large proteins and protein complexes in an environment that closely mimics that of a cell. Proteins are the molecules that carry out many of the biological functions underlying normal health and disease. In many instances, disease-causing mutations alter a protein's function by disrupting its three-dimensional structure. Thus, an important part of biomedical research is to understand the relationships between the structure and biological function of proteins.

In this study, researchers used a novel combination of two biophysical techniques—X-ray scattering and nuclear magnetic resonance (NMR) spectroscopy—that provide complementary information on the size and shape of the protein complex and the relative orientations of the different components of the complex. By integrating the two different types of data with structural knowledge of the individual components, the researchers were able to determine how two bacterial proteins—Enzyme I and HPr—fit together to form a large molecular complex that regulates the transport of sugar molecules in and out of the cell. In addition, by comparing the structure of the complex with that of Enzyme I alone, the scientists found that the enzyme changes shape when it joins with HPr; this change in shape may be important for the enzyme to function properly. Since X-ray scattering or NMR alone would have been insufficient for characterizing the structure of this enzyme system, the combined use of the techniques provides an important new strategy for determining the size, shape, and orientation of large proteins and protein-protein complexes. The application of this new approach for studying the structural properties of large proteins and protein complexes will provide important information on their biological functions in health and disease.

Schwieters CD, Suh J-Y, Grishaev A, Ghirlando R, Takayama Y, and Clore GM. Solution structure of the 128 kDa enzyme I dimer from Escherichia coli *and its 146 kDa complex with HPr using residual dipolar couplings and small- and wide-angle X-ray scattering. J Am Chem Soc 132: 13026-13045, 2010.*

Timing How Fast Proteins Fold, One Molecule at a Time: Scientists in NIDDK's Intramural Research Program have applied recently developed statistical methods to determine how fast individual protein molecules "fold" into their correct biological shapes. When a protein is made in a cell, it folds into a uniquely defined three-dimensional structure. This "native" structure, as it is called, is intimately linked to the protein's function. In cases where a protein's native structure is disrupted, such as by genetic mutations that alter its ability to fold properly, the resulting impairment of the protein's normal function can cause disease. Given the importance of protein structure and function in normal health and disease, scientists are interested in understanding the physical process that leads to the formation of a protein's correct native structure.

NIDDK intramural scientists have previously pushed the limits of a technique called single-molecule fluorescence resonance energy transfer (FRET) to observe the transitions between unfolded and native states during the folding process of individual protein molecules. To do this, they labeled proteins with dye molecules that emit light of different colors depending on whether the protein is folded or unfolded. These researchers have now analyzed the FRET data using new statistical methods, developed by another team of scientists in the Intramural Research Program, to determine how much time the protein molecules spend in the unfolded state and the native state. This type of data analysis also provides information on the percentage of molecules in the folded and unfolded states under the experimental conditions, as well as information on the overall shape of the molecules in each state. In addition, the scientists applied a new computational procedure to identify the transitions between the unfolded and native states. As all of the important structural changes associated with going from the unfolded state to the native state occur during these transitions, the application of this new methodology may allow researchers to uncover the fundamental mechanisms that guide the folding process and identify the misfolding steps that are often associated with diseases.

Chung HS, Gopich IV, McHale K, Cellmer T, Louis JM, and Eaton WA. Extracting rate coefficients from single-molecule photon trajectories and FRET efficiency histograms for a fast-folding protein. J Phys Chem A doi: 10.1012/jp1009669, 2010.

THE HUMAN GENOME ARCHITECTURE

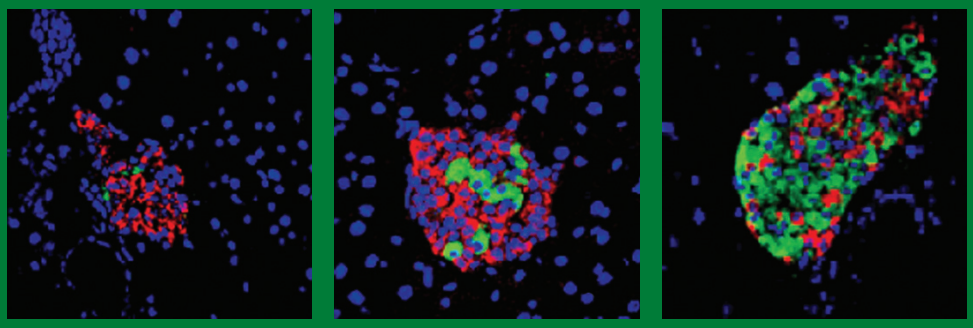
Mapping the Three-Dimensional Architecture of the Human Genome: Using a chemical linkage strategy combined with a large-scale DNA sequencing effort, scientists have revealed how DNA is organized within cells. Genomic DNA exists as a set of separate segments that are packaged along with other components into units called chromosomes. Within a cell, the chromosomes are found in a specific compartment, the nucleus. Generally, chromosomes are in a dense, dynamic clump. Scientists have been trying to understand how the chromosomes are organized in the nucleus. Previous studies indicate that chromosomes occupy distinct "territories" within the nucleus. Within territories, chromosomes are flexible such that the DNA portion of one part of a chromosome can interact with the DNA portion of another chromosome, and DNA at one end of a chromosome can interact with DNA at the other end. A technique developed earlier has allowed scientists to determine all the inter- and intra-chromosomal interactions between chosen areas of the genome.

Building on this technique, investigators developed a new method, which they call Hi-C, to enable mapping of inter- and intra-chromosomal interactions throughout the entire genome. Data from this technique provided insight into how the genome is organized in the nucleus. The data confirmed the concept of "chromosome territories" and that small, gene-rich chromosomes tend to be near each other physically. Furthermore, the scientists discovered that the chromosomes could be categorized into two compartments that were physically separated. One compartment contained active genes while the other compartment contained inactive genes.

The scientists propose that their data are consistent with a fractal model—one that has the same pattern no matter how close one zooms in. In this model, the

chromosomes are densely folded in the nucleus and highly compact. In addition, there are no knots in this model. Previously suggested models for chromosome organization contained knots that did not offer the flexibility that might be necessary for genes to turn on and off. These findings and this new technique will allow scientists to look even more closely at genome-wide chromosome interactions and learn more about how chromosomes are organized, including whether genome shape is altered across cell types. Evidence suggests that genome structure affects the turning on and off of genes. Therefore, understanding genome structure will provide insight into how the shape of the genome affects health and disease.

Lieberman-Aiden E, van Berkum NL, Williams L, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326: 289-293, 2009.



Research highlighted in this chapter demonstrates that pancreatic glucagon-producing alpha cells could convert to become insulin-producing beta cells. These images show alpha cells (red), beta cells (green), and other pancreatic cell types (blue). The image on the left shows a pancreas from a diabetic mouse containing only a few green beta cells. Over time, the mouse regenerated its beta cells, as shown by more green beta cells after 1 month (middle panel) and 10 months (right panel). Importantly, the researchers found that the new beta cells arose from alpha cell conversion. Identifying ways to replace insulin-producing beta cells is important for both type 1 and type 2 diabetes, and this new insight about the ability of one pancreatic cell type to convert to another can inform future research toward developing cell-based therapies for people with diabetes.

Images provided by Dr. Pedro L. Herrera and reprinted by permission from Macmillan Publishers Ltd: Nature 464: 1149-1154, copyright 2010.

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 25.8 million people in the U.S.—or 8.3 percent of the total population—and is the seventh leading cause of death.[1] Diabetes lowers average life expectancy by up to 15 years,[2] increases cardiovascular disease risk two- to four-fold, and is the leading cause of kidney failure, lower limb amputations, and, in working-age adults, blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2007—including costs of medical care, disability, and premature death—was $174 billion.[1] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.[1]

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.

Type 1 diabetes affects approximately 5 percent of individuals with diagnosed diabetes.[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 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, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels to near the normal levels achieved by functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working to develop beta cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diabetes cases in the U.S.[1] Type 2 diabetes is associated with several factors, including older age and a family history of the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.[3] Type 2 diabetes occurs at elevated rates among minority groups, including African Americans, Hispanic Americans, American Indians, and Native Hawaiians.[1]

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin.

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[1] *2011 National Diabetes Fact Sheet. Centers for Disease Control and Prevention. Atlanta, GA.*

[2] *Portuese E and Orchard T: Mortality in Insulin-Dependent Diabetes. In* Diabetes in America *(pp. 221-232). Bethesda, MD: National Diabetes Data Group, NIH, 1995.*

[3] *Eberhardt MS, et al: MMWR 53: 1066-1068, 2004.*

As a result, blood glucose levels rise, and at first the pancreas produces more insulin to compensate. Gradually, however, the pancreatic beta cells lose their capacity to secrete insulin, and the timing of insulin secretion becomes abnormal. 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 79 million adults in the U.S. who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[1] This population is at high risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with pre-diabetes can dramatically reduce their risk of developing type 2 diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight. Moreover, follow-up research has shown that this benefit of reduced diabetes risk can persist for at least 10 years.

Type 2 diabetes was previously called "adult-onset" diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. First, the onset and severity of disease complications correlate with the duration of diabetes; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. Second, 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 diabetes in offspring. Thus, the rising rates of diabetes and pre-diabetes in young women could lead to a vicious cycle of ever-growing rates of diabetes. Third, diabetes often becomes more difficult to control over time. With longer duration of disease, patients may find it increasingly difficult to strictly control their blood glucose levels and thus prevent or delay the development of complications. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the U.S.

The NIDDK is supporting research to better understand metabolism and the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the Institute'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 Institute is vigorously pursuing studies of prevention and treatment approaches for these diseases.

GENETICS OF DIABETES

Mapping Gene Regulatory Sites To Understand Islet Biology and Diabetes: Scientists have developed a new technique for identifying regions of the genome that influence gene activity and generated insight into how a specific sequence variant can affect risk for type 2 diabetes. Regions of the genome called "open chromatin" contain genes and regulatory DNA elements that are actively being used within a particular cell type. Using a new technique, the investigators isolated open chromatin from human pancreatic islet cells, which produce insulin and other important hormones, and created a map of these sites in the genome. They hypothesized that islet-specific open chromatin was likely to contain sequences that influence the activity of islet-specific genes and, therefore, sequences that may be associated with diabetes risk. They found that most islet-specific open chromatin sites were in or near genes with known functions in islets. In addition, they compared previously identified type 2 diabetes-associated DNA sequence variants to their map and discovered that a number of disease-associated variants are linked to islet-specific open chromatin sites.

Notably, a variant in the gene *TCF7L2*, which has been consistently associated with type 2 diabetes across diverse ethnic groups, was determined to overlap with an islet-specific open chromatin site. This suggests that the disease-associated variation may regulate the activity of the gene since it is not within the part of the gene that codes for a protein. The scientists then demonstrated that the risk-associated sequence variant was more likely than the non-risk sequence variant to be found in open chromatin, meaning that people with the risk version may produce more of the protein encoded by *TCF7L2*. Indeed, they found that the risk-associated variant can affect gene activity. These results suggest that the risk variant may affect activity of *TCF7L2* by

opening the site to allow more of the protein to be made and provide a potential mechanism for type 2 diabetes susceptibility.

The islet-specific map generated in this study provides a new tool for understanding the regulation of genes important for islet cell biology and for narrowing genomic locations likely to harbor unidentified sequence variants that influence type 2 diabetes susceptibility. This study also validates a new technique for identifying regions of the genome that regulate gene activity. The technique provides an additional means to move beyond identification of disease-associated sequence variants to an understanding of their influence on disease risk, particularly for variants that do not affect the code for a protein. Determining the mechanism by which genetic factors contribute to diabetes is key to understanding both type 1 and type 2 diabetes, identifying individuals at risk, developing and testing prevention strategies, and generating more personalized interventions for people with or at risk for disease.

Gaulton KJ, Nammo T, Pasquali L, et al. A map of open chromatin in human pancreatic islets. Nat Genet 42: 255-259, 2010.

New Discoveries on the Genetics of Blood Glucose Regulation and Insulin Resistance: New genomic technologies have provided a wealth of data on the complex genetic underpinnings of diseases like type 2 diabetes. For example, researchers have used a technology called the genome-wide association (GWA) study to compare single nucleotide polymorphisms (SNPs) throughout the genomes of thousands of people with and without the disease to identify common variants that affect the likelihood of developing diabetes. Recently, researchers took a slightly different approach to shed still more light on diabetes genetics. In people without diabetes, pancreatic function tightly controls the level of glucose present in the blood, ensuring that there is always enough glucose that cells will have an adequate supply, but not so much that the excess is toxic. The new research, however, proceeds from the observation that, even among people who do not have diabetes, there is variation in blood glucose and insulin levels.

For this study, a consortium of investigators re-examined data from dozens of prior GWA analyses, focusing on the "control" populations—those without diabetes. By grouping the participants according to several different measures of metabolic function, they identified a total of 16 genetic locations that appear to have an effect on fasting blood glucose levels, and two that influence fasting insulin levels and insulin resistance, all in people without diabetes. Four of these genetic locations previously had been associated with type 2 diabetes. This study also analyzed potential associations of the variants with type 2 diabetes, and found that five of the 16 gene regions also are linked to risk for the disease. This collaborative study helps define a new approach to identify diabetes risk genes. Further analysis of the genes near the variations found in these studies will help better explain how blood glucose levels are controlled in health and disease. By better understanding the molecular control of healthy blood glucose levels, scientists may one day be more able to predict type 2 diabetes with precision, tailor treatment to people in particular risk categories, and develop improved therapies to help people with diabetes keep their blood glucose at an appropriate level.

Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42: 105-116, 2010.

Identification of Novel Genetic Risk Factor for Type 1 Diabetes Using Analysis of Gene Networks: Scientists have identified a novel genetic variant associated with type 1 diabetes risk that regulates a network of immune system genes. GWA studies have identified many common genetic variants that are associated with human disease. For many of these variants, however, it is not known how the specific variation affects disease risk. This may be true particularly if the variant is not located in a gene or the variant does not appear to affect the activity of the gene in which it is located. To translate these associations with disease to discovery of their roles in disease risk, scientists are investigating whether "gene networks"—sets of genes that are co-regulated—are affected by these genetic variants. In the cell, genes that have related functions tend to be co-regulated; that is, they are turned on or off in similar ways with comparable timing.

In this study, scientists analyzed data from rat tissues to identify gene networks and correlate changes in the regulation of these networks with genetic variants.

They identified a novel gene network that includes a number of genes involved in the immune response to viruses. This network is regulated by the protein factor Irf7 and designated the Irf7-driven inflammatory gene network (IDIN). The scientists demonstrated that the rat IDIN was influenced by a genetic variation on chromosome 15 in a gene known as *Ebi2*, which encodes a protein involved in migration of a specific type of immune cell.

To translate this finding to humans, the scientists looked to see whether the IDIN similarly exists in human immune cells. Not only did they discover the human *IRF7*-driven network, but they also found that genetic variants affecting the human form of *EBI2* had an effect on the IDIN gene network. Since the human IDIN contained a well-characterized type 1 diabetes susceptibility gene, the researchers hypothesized that other genes in the IDIN might be associated with type 1 diabetes risk. They found that genetic variants near IDIN genes were more likely to be associated with type 1 diabetes than were genes not in the network, and they identified a new genetic variant for the disease near *EBI2*. Therefore, this exciting study implicated IDIN genes and their regulation in type 1 diabetes and elucidated the role of *EBI2* in disease risk. Uncovering the functional effects of genetic variation associated with type 1 diabetes will not only increase understanding of how this disease develops, but will illuminate key targets for the development of therapies to prevent the disease.

Heinig M, Petretto E, Wallace C, et al. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature 467: 460-464, 2010.

BETA CELLS AND DIABETES

Protein Found That Drives Development of Insulin-Producing Cells: Finding ways to reduce or eliminate the burden of injected insulin therapy for people with type 1 diabetes and some with type 2 diabetes is an important goal of diabetes research. One approach to eliminating the dependency on injected insulin is to replenish a person's insulin-producing beta cells. Stem cells or other types of cells that could be reprogrammed to produce insulin may represent a good source of replacement tissue, but to tap their potential it is critical to understand the developmental program that creates a functional beta cell. New research uncovered a key factor necessary for making insulin-producing beta cells in both humans and mice. Previous research had identified a protein that helps trigger embryonic development of pancreatic islets, which contain beta cells and other cell types. Scientists now have found another key protein needed for the subsequent development of distinct islet cell subtypes. Mice lacking the newly identified protein—called Rfx6—can make islets, but these islets do not contain insulin-producing cells. They also fail to make some other hormones normally made by the pancreas. Interestingly, the scientists also found that a rare form of neonatal diabetes is associated with mutations in the human gene that produces the Rfx6 protein, suggesting that Rfx6 plays a critical role in beta cell development in humans as well as mice. Researchers now know they will have to ensure that Rfx6 is present in order to successfully generate beta cells from some other cell type for transplantation into people with diabetes.

Smith SB, Qu H-Q, Taleb N, et al. Rfx6 directs islet formation and insulin production in mice and humans. Nature 463: 775-780, 2010.

Alpha Cells Take on New Identity To Overcome Diabetes: Researchers discovered that pancreatic glucagon-producing alpha cells could convert to insulin-producing beta cells in a mouse model of diabetes. Beta cells are destroyed by the immune system in people with type 1 diabetes and may not function normally in people with type 2 diabetes. Identifying ways to replace beta cells to restore the body's insulin-producing capacity would benefit people with type 1 or type 2 diabetes, and is a major goal of research.

Toward this goal, scientists examined beta cell regeneration in a mouse model of diabetes. Using a combination of genetic engineering and chemical treatment, the researchers destroyed nearly all of the beta cells in the mice, resulting in the development of diabetes in the animals and dependency on injected insulin for their survival. The scientists found that, over time, the beta cells regenerated; after 5 months, the animals produced enough of their own insulin to survive without external insulin treatment. Further experiments showed that the source of the new beta cells was the

alpha cells, rather than, for example, the few remaining beta cells. This insight suggests that it may be possible to develop therapies to promote conversion of alpha cells to beta cells to restore insulin production in people with diabetes. Further research is needed to determine if this conversion of one pancreatic cell type to another can occur in humans, and how to protect new beta cells from immune system attack in type 1 diabetes. But, the finding opens up intriguing new avenues for research toward cell replacement therapy for diabetes.

Thorel F, Népote V, Avril I, et al. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 464: 1149-1154, 2010.

Regulating a Regulator of Pancreas Development: New research has shed light on the regulation of a key protein involved in pancreas development and function. The protein, Pdx1, is required for pancreas development in mice and humans; mutations in the *Pdx1* gene, which can decrease the levels of Pdx1 protein, are associated with diabetes in humans. Because proper amounts of Pdx1 protein are important for its role in the pancreas, scientists examined how levels of the protein are controlled. By studying cells grown in the laboratory, they discovered that another protein, Pcif1, targets Pdx1 for degradation. To understand what effect this has in the whole animal, they examined mice that were genetically engineered to have low levels of Pdx1. These mice had several conditions associated with diabetes or risk for the disease, including impaired glucose tolerance and insulin secretion, as well as reduced mass of their insulin-producing beta cells. Using genetic techniques, the scientists demonstrated that if they also reduced levels of Pcif1 in these mice, levels of Pdx1 increased to normal—because not as much Pcif1 was present to target Pdx1 for degradation. This normalization was associated with improved beta cell survival and increased beta cell mass, which in turn led to improved glucose tolerance and insulin secretion. In other words, signs of diabetes observed in mice with reduced Pdx1 levels were relieved by concurrent reduction of Pcif1. The research provides new insights into how Pdx1 protein levels are controlled and suggests that Pcif1 regulation of Pdx1 could be a therapeutic target for treating diabetes.

Claiborn KC, Sachdeva MM, Cannon CE, Groff DN, Singer JD, and Stoffers DA. Pcif1 modulates Pdx1 protein stability and pancreatic beta cell function and survival in mice. J Clin Invest 120: 3713-3721, 2010.

ADVANCING TECHNOLOGY TO MANAGE DIABETES

Continued Progress in Glucose Monitoring Technology—Implantable Glucose Sensor: New research is paving the way toward less burdensome glucose monitoring for people with diabetes. People with type 1 diabetes must monitor their blood glucose levels and administer insulin to keep glucose levels in a healthy range. Day-to-day monitoring is most commonly achieved through multiple daily finger sticks alone; some people now also use continuous glucose monitors in combination with finger sticks. While these methods are valuable tools for helping people with diabetes stay healthy, they entail a fair amount of patient effort and discomfort. Therefore, researchers are working to advance technologies that can minimize patient burden while sustaining the health benefits of frequent glucose monitoring.

In a recent report, a team of bioengineers described testing of an implantable glucose sensor that monitors tissue glucose and reports data to an external wireless receiver. When implanted into pigs, the system functioned continuously for over a year. The implanted sensor also worked when tested for several months in pigs that were made diabetic through administration of a chemical that is toxic to the insulin-producing pancreatic beta cells. These experiments showed that the sensor remained accurate whether glucose levels were high, low, or normal, and helped gauge the speed with which it detects rising or falling blood glucose. The scientists plan to conduct clinical trials to test the sensor in people. The system does not automatically deliver insulin, so patients would still need to administer insulin based on the sensor readings. However, these results are encouraging because an implantable device not only could reduce the need for finger sticks, but also potentially be used in the future as part of an "artificial pancreas" to automate glucose sensing and insulin delivery.

Gough DA, Kumosa LS, Routh TL, Lin JT, and Lucisano JY. Function of an implanted tissue glucose sensor for more than 1 year in animals. Sci Transl Med 2: 42ra53, 2010.

AUTOIMMUNITY IN TYPE 1 DIABETES

It's All in the Presentation—Type 1 Diabetes and the Display of Insulin to Immune Cells: Scientists discovered that a variant of an immune system molecule may contribute to type 1 diabetes by enabling an aberrant immune reaction against insulin. Genetic variation in the *HLA* genes, which encode a key immune recognition protein, accounts for a large proportion of the genetic risk for type 1 diabetes in humans. However, little is known about how this variation leads to autoimmunity, in which T cells of the immune system destroy the insulin-producing beta cells in the pancreas, resulting in type 1 diabetes. Previous research identified a specific fragment of the insulin molecule as important to the development of type 1 diabetes in a mouse model of the disease. The immune recognition protein binds this fragment and "presents" it to T cells. If T cells recognize insulin presented in this way in an organ called the thymus, then those cells are destroyed because the body normally tries to prevent immune reactions against itself. If T cells that recognize "self" are not destroyed in this process, then they are released throughout the body and could attack the insulin-producing beta cells.

By studying how the specific insulin fragment binds to different sites on the variant immune recognition protein in mice, the scientists noted a surprising finding. Rather than observing strong binding between this variant immune protein and the insulin fragment, as had previously been suggested, they observed that weaker binding led to activation of T cells involved in autoimmunity. The scientists speculated that this weaker binding did not permit adequate presentation of the insulin fragment to the T cells in the thymus and therefore allowed the T cells to escape the normal mechanism that should have destroyed them. Further research is necessary to determine whether a similar mechanism is associated with type 1 diabetes in humans. If it is, then this finding could present an exciting opportunity to intervene in the immune process to prevent the disease or slow its progression.

Stadinski BD, Zhang L, Crawford F, Marrack P, Eisenbarth GS, and Kappler JW. Diabetogenic T cells recognize insulin bound to IA^{g7} in an unexpected, weakly binding register. Proc Natl Acad Sci USA 107: 10978-10983, 2010.

Novel Immune Target for Preventing or Treating Type 1 Diabetes: Researchers in a clinical trials network—Type 1 Diabetes TrialNet—reported that a drug that destroys immune system cells called B lymphocytes preserved the function of insulin-producing beta cells in people newly diagnosed with type 1 diabetes for 1 year. Scientists have known that type 1 diabetes is caused by aberrant immune system destruction of insulin-producing cells, and have implicated immune cells called T lymphocytes (or T cells) in this attack. It had not been clear, however, whether B lymphocytes (or B cells) were involved or could be targets for therapeutic approaches. Scientists tested whether destroying B lymphocytes with four separate infusions of the drug rituximab shortly after type 1 diabetes diagnosis could slow progression of the disease. After 1 year, people who received the drug produced more insulin, had better control of their diabetes, and did not have to take as much insulin to control their blood glucose levels compared to people receiving placebo. However, at 2 years, the effect of the treatment dissipated. Because of the side effects associated with this immunosuppressive drug, the risk to benefit ratio would not suggest that rituximab be used as a therapy for people with type 1 diabetes. Nonetheless, the finding is very important because it demonstrates that B lymphocytes may be a key target for type 1 diabetes prevention or treatment. Rituximab treatment results in a general depletion of B lymphocytes, and the effect of the drug was lost when the depletion ended; it would be unhealthy for B lymphocytes to be chronically depleted (through additional infusions of the drug) because these cells are a part of a functioning immune system. Thus, this study suggests that it may be possible to prevent or treat type 1 diabetes more safely by identifying ways to target the specific B lymphocytes involved in the disease without depleting B lymphocytes more generally.

Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med 361: 2143-2152, 2009.

COMBATING TYPE 2 DIABETES RISK FACTORS IN YOUTH

Results of a Middle School Intervention To Lower Risk of Diabetes: New research shows that

an intervention program in middle schools lowered the obesity rate in a group of students at particularly high risk for type 2 diabetes, but did not have a greater impact on the overall rate of obesity and overweight than was observed in control schools. Type 2 diabetes is an emerging health problem in youth, particularly minority youth, being driven by the obesity epidemic.

To address the problem, the NIDDK-led HEALTHY clinical trial examined whether a middle school-based intervention could lower risk factors for type 2 diabetes. The study was conducted in schools with a high enrollment of minority children and youth from low-income families. Conducted for 3 years—from the beginning of the sixth grade to the end of the eighth—the HEALTHY study involved 4,603 students attending 42 U.S. middle schools in seven areas of the country. Each school was randomly assigned to implement the intervention program or to serve as a comparison (control) school. The intervention program involved changes in school food services; longer, more intense periods of physical education; and classroom activities to promote healthful behavior changes.

The intervention was found to lower the obesity rate in students who started out overweight or obese in sixth grade, a group of children who would otherwise be at higher risk of future type 2 diabetes than the others in the study. Surprisingly, however, schools that implemented the program did not differ from comparison schools in the study's primary outcome—the combined prevalence of overweight and obesity—which had declined by 4 percent in both the intervention and control schools by the end of the 3-year study. One possible explanation is that comparison schools may have independently implemented healthful changes to the school environment because of increased awareness about the problem of childhood obesity fostered by the study. Future research will examine what changes in policy may have been implemented in the comparison schools. The HEALTHY results are important for informing future school-based efforts to reduce overweight and obesity in children.

The HEALTHY Study Group. A school-based intervention for diabetes risk reduction. N Engl J Med 363: 443-453, 2010.

TESTING TREATMENT APPROACHES FOR TYPE 2 DIABETES

Inexpensive, Generic Drug Improves Blood Glucose Control in People with Type 2 Diabetes: Researchers have discovered that the drug salsalate helped people with type 2 diabetes control their blood glucose levels. Salsalate is an inexpensive, generic anti-inflammatory drug that is chemically similar to aspirin, but causes fewer stomach problems. It has been used safely for decades to treat people with arthritis. Because research is showing that metabolic conditions, including type 2 diabetes, are associated with chronic inflammation, scientists tested whether this anti-inflammatory drug could effectively treat people with type 2 diabetes. In the first phase of the Targeting INflammation with SALsalate in Type 2 Diabetes (TINSAL-T2D) clinical trial, 108 people were randomly assigned to four different treatment regimens: one group received placebo, and three groups received different doses of the drug. All participants continued their regular diabetes treatment regimen during the trial. After 3 months, people taking salsalate had lower blood glucose and triglyceride levels on average compared to people taking placebo. Some participants experienced adverse changes such as increased excretion of protein in the urine and higher levels of LDL (bad) cholesterol. Thus, researchers are conducting a longer, larger trial to further test salsalate—knowledge that is needed to further evaluate the relative benefits and risks of the drug. With more research, salsalate may prove to be an inexpensive way to help treat the millions of people with type 2 diabetes in the U.S.

Goldfine AB, Fonseca V, Jablonski KA, et al. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152: 346-357, 2010.

Intensive Lifestyle Intervention Reduces Risk Factors for Cardiovascular Disease in People with Type 2 Diabetes: In a major ongoing NIDDK-supported clinical study, researchers showed that after 4 years, an intensive lifestyle intervention (ILI) program reduces cardiovascular disease (CVD) risk factors in overweight and obese people with type 2 diabetes. Although the short-term benefits of lifestyle interventions to achieve weight control are well understood, long-term effects of intervention on

blood glucose (glycemic) control and CVD risk factors have not been extensively studied. The nationwide Look AHEAD (Action for Health in Diabetes) trial is investigating the long-term health effects of a sustained ILI program, as compared to the current diabetes support and education (DSE) strategies, on improving cardiovascular outcomes. More than 5,000 overweight or obese participants between the ages of 45 and 76, with type 2 diabetes, were randomly assigned to either the ILI or DSE strategy. Individuals in the DSE group were invited to three group sessions per year that provided information conveying the importance of controlled diet, nutrition, and physical activity, as well as social support sessions. In addition to the information provided to the DSE group, individuals in the ILI group received defined, reduced caloric intake and physical exercise goals, frequent weighing, and specific behavior modification instruction in self-monitoring (such as maintaining a diet and exercise diary), problem solving, and goal setting. The participants receiving ILI also had more frequent group meetings, as well as regular one-on-one lifestyle counseling.

The Look AHEAD study had previously reported beneficial health effects after 1 year, and the investigators have now found that participants in both groups showed positive changes in their health over 4 years. On average, across all 4 years, participants in the ILI group lost significantly more weight than those in the DSE group (6.2 percent vs. 0.9 percent reduction). ILI group members also experienced improved fitness, glucose control, blood pressure, and HDL (good) cholesterol. Both groups showed reductions in LDL (bad) cholesterol, but the reductions were larger in the DSE group because of their greater use of cholesterol-lowering medications.

A vast majority of participants remain in the study after 4 years, and the trial is planned to continue for up to 13.5 years. These findings reveal a strong link between intensive lifestyle intervention and improvements in CVD risk factors associated with overweight, obesity, and diabetes. Longer-term results from this ongoing trial will determine whether these effects can be sustained and whether they will ultimately lead to reduced incidence of illness and death from CVD, as well as other health benefits.

The Look AHEAD Research Group. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med 170: 1566-1575, 2010.

New Insights into Treating People with Type 2 Diabetes and a High Risk of Heart Disease:

Results from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial are providing important evidence to help guide treatment recommendations for adults with type 2 diabetes who have had a heart attack or stroke or who are otherwise at high risk for heart disease. ACCORD investigators studied over 10,000 adults who had type 2 diabetes for an average of 10 years and were at especially high risk for cardiovascular events. All participants were enrolled in the ACCORD blood glucose treatment clinical trial; participants were also enrolled in either the blood pressure trial or the lipid trial.

Results of the ACCORD blood glucose trial were reported in 2008 and showed that intensively lowering blood glucose to near-normal levels brought a higher risk of death for participants than standard blood glucose control. More recently, results of the lipid and blood pressure trials were announced. In the blood pressure trial, researchers randomly assigned participants with elevated blood pressure to a target systolic blood pressure—the top blood pressure number—of either less than 140 (the standard group) or a "normal" level of less than 120 (the intensive group). The study found that lowering blood pressure to normal levels did not significantly reduce the risk of cardiovascular events overall, although it may reduce the risk of stroke. However, this treatment strategy was associated with more complications, such as abnormally low blood pressure. In addition, some laboratory measures of kidney function were worse in the intensive therapy group, but there was no difference in the rates of kidney failure. In the lipid trial, researchers compared two groups. One group received a fibrate medication, which lowers triglycerides and raises HDL (good) cholesterol, while the other group did not. Both groups received a statin medication, which lowers LDL (bad) cholesterol. Combination therapy of statin and fibrate medications appeared to be safe, but did not lower the risk of heart attack, stroke, or death from heart disease more than the statin alone.

In addition to examining the effects of the different therapies on macrovascular (large vessel) damage to the heart, ACCORD researchers also studied the effect of these therapies on microvascular (small vessel) damage to organs and tissues. Intensive blood glucose control was found to reduce some indicators of eye, nerve, and kidney disease compared to standard control, but the intensive and standard control groups did not differ in the rate of progression to kidney failure, nerve disease, and major vision loss. The results are consistent with findings from the ACCORD Eye Study, which found that, in a subset of participants, intensive blood glucose control reduced progression of a form of eye disease called diabetic retinopathy. The scientists measured less severe eye damage in the Eye Study and saw a benefit, but there was no benefit observed with respect to more severe damage—major vision loss—in the full trial. The Eye Study also found that adding a fibrate drug to statin therapy for control of blood lipids reduced progression of diabetic retinopathy. However, intensive blood pressure control provided no additional benefit compared with standard blood pressure control.

Although the ACCORD finding of increased mortality risk outweighed the benefits of near-normal glucose control in the participants of this trial, it remains possible that more intensive blood glucose control may be more beneficial earlier in the course of type 2 diabetes than was studied in ACCORD. Optimal therapy for older patients newly diagnosed with diabetes or without complications will have to be tested in future research. The ACCORD results are helping health care providers tailor therapy for their patients with type 2 diabetes, by considering the risks and benefits of different treatment approaches for each patient.

ACCORD Study Group, Ginsberg HN, Elam MB, et al. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med 362: 1563-1574, 2010.

ACCORD Study Group, Cushman WC, Evans GW, et al. Effects of intensive blood-pressure control in type 2 diabetes mellitus. N Engl J Med 362: 1575-1585, 2010.

Ismail-Beigi F, Craven T, Banerji MA, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet 376: 419-430, 2010.

ACCORD Study Group; ACCORD Eye Study Group, Chew EY, et al. Effects of medical therapies on retinopathy progression in type 2 diabetes. N Engl J Med 363: 233-244, 2010.

REGULATORS OF METABOLISM IN HEALTH AND DISEASE

Molecular Insights Could Lead to Improved Type 2 Diabetes Drugs: Researchers have identified the molecular mechanism by which the master regulator of fat cell biology and development, a protein called PPAR-gamma, regulates insulin sensitivity. A hallmark of type 2 diabetes is that cells become resistant, or less sensitive, to the action of insulin, a hormone that promotes the uptake of glucose from the bloodstream into the cells. Some type 2 diabetes drugs have been developed to target PPAR-gamma; the drugs make the body more sensitive to insulin, but come with unwanted side effects, such as weight gain and an increased risk of heart failure. In this study, scientists discovered that a specific chemical modification (phosphorylation) to PPAR-gamma leads to the abnormal regulation of a number of genes related to obesity and insulin sensitivity in mice. They also found that the diabetes drugs block this modification in people with type 2 diabetes, thus countering insulin resistance. The drugs also broadly stimulate PPAR-gamma, which may be responsible for the negative side effects. The research suggests that a new generation of type 2 diabetes drugs could be designed to block the chemical modification only, without broadly stimulating PPAR-gamma, to improve insulin sensitivity without the unwanted side effects.

Choi JH, Banks AS, Estall JL, et al. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPAR gamma by Cdk5. Nature 466: 451-456, 2010.

Understanding How Cells Relieve Stress—Implications for Diabetes: Scientists uncovered how the Wolfram syndrome 1 (WFS1) protein protects cells, including pancreatic beta cells, from an uncontrolled response to a type of cellular stress. Mutations in the gene encoding WFS1 cause Wolfram syndrome, a genetic form of diabetes also associated with optic atrophy, neurodegeneration, and psychiatric illness. Diabetes resulting from Wolfram syndrome is characterized by non-immune mediated loss of the insulin-producing beta cells. Beta cell death in

Wolfram syndrome has been suggested to result from an uncontrolled response to a type of stress in a cellular component called the endoplasmic reticulum (ER). One of the critical processes that occur in the ER is the process of "protein folding" by which a protein acquires its mature structure. Stress on the ER, whether caused by protein misfolding or other various stimuli, can lead to detrimental consequences. In response to this stress, the cell instigates a response which ultimately leads to the production of proteins that relieve the stress. This response, however, must be mitigated once the stress is alleviated because hyperactivation of the stress response can lead to cell death.

WFS1 had previously been shown to mitigate the ER stress response in cells, but in a new study, scientists revealed the specific role WFS1 plays in the stress response. Using rodent and human cells, the scientists determined that WFS1 associates with another protein—ATF6alpha—a key regulator of the response to ER stress. They found that WFS1 suppressed ATF6alpha activity by recruiting it to a protein degradation complex, thus lowering levels of ATF6alpha and keeping the stress response restricted. In response to a stress signal, however, the interaction between WFS1 and ATF6alpha was disrupted, freeing ATF6alpha to turn on genes whose protein products function to relieve the ER stress. When the scientists depleted WFS1 from laboratory-grown cells, however, they observed that ATF6alpha levels were increased, and the stress response was chronically hyperactivated. Moreover, beta cells from mice genetically engineered to lack WFS1 and other types of cells (lymphocytes) from patients with Wolfram syndrome similarly exhibited abnormally high amounts of ATF6alpha.

These results indicate that WFS1, by working through ATF6alpha, has a critical role in regulating the ER stress response and in preventing cells from dysfunction and cell death caused by a hyperactive stress response. Interestingly, variants in the gene encoding WFS1 are also associated with type 2 diabetes. This research suggests that WFS1 could be a key target for strategies to treat or prevent diseases related to ER stress, including diabetes.

Fonseca SG, Ishigaki S, Oslowski CM, et al. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. J Clin Invest 120: 744-755, 2010.

Stoking the Fat Furnace with SIRT3: Researchers have uncovered a new mechanism regulating how cells burn fat. Fat tissue holds the body's major energy reserve. When the body needs to tap into this reserve, such as during a fast, fat molecules are mobilized to other organs and tissues. There, mitochondria—the "powerhouses" of the cell—can switch from burning glucose, the primary cellular fuel, to burning fat for energy. New research suggests that a protein called SIRT3 regulates this important switchover in metabolism. In a series of experiments, scientists compared metabolism between normal mice and mice genetically engineered to lack SIRT3, under both fed and fasted conditions. They found that during a fast, mice lacking SIRT3 had incomplete fat-burning in their livers, resulting in abnormally high levels of fat intermediates and triglycerides. Moreover, while mice with and without SIRT3 both appeared normal in the fed state, mice lacking SIRT3 showed symptoms of fat-burning disorders when challenged with specific metabolic stresses. For example, when fasted, these mice produced less energy in their livers and had low tolerance to cold. SIRT3 activates other proteins, such as metabolic enzymes, by removing specific chemical modifications called acetyl groups. Molecular experiments revealed that a key enzyme in fat-burning is regulated by these modifications and needs SIRT3 in order to be activated during a fast. Because SIRT3 itself depends on a molecule whose levels reflect the cell's metabolic state, the scientists hypothesize that SIRT3 acts as a "metabolic sensor," enabling cells to quickly switch to fat-burning in response to energy needs. Defects in fat-burning are associated with diabetes and other metabolic disorders. Researchers can now explore the possible pathogenic role of SIRT3 in metabolic disorders and the potential therapeutic value of boosting SIRT3 activity.

Hirschey MD, Shimazu T, Goetzman E, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464: 121-125, 2010.

Candidate Coordinator for Inflammation and Metabolic Diseases: A molecule that helps the body fight viral infections may also play an important role in metabolic diseases. Research over the past decade demonstrated that inflammation, normally one of the body's defenses against invading microbes, can contribute to very different processes—the development

and progression of chronic metabolic conditions such as obesity and diabetes. How and why this happens is being actively investigated so that new therapeutic and preventive approaches may be developed. Contemplating how inflammation might connect the seemingly diverse conditions of infection and metabolic disease, scientists have speculated that perhaps a molecule(s) governing infection-triggered inflammation is also stimulated when the body has excess nutrients, as in obesity. New research suggests that a molecule called double-stranded RNA-dependent protein kinase, or PKR, could be a candidate. Known previously for its important role in detecting viral invasion and orchestrating antiviral responses in a cell, PKR is also associated with an inflammatory signaling pathway that has been implicated in insulin resistance—a condition associated with type 2 diabetes and obesity.

To determine whether PKR coordinates activities that disrupt metabolism, scientists studied PKR in mouse models and cells. These studies revealed that PKR is indeed activated in response to nutrient excess and other metabolic stresses and can inhibit a key component of insulin signaling, both directly and by activating the inflammatory pathway. Moreover, in experiments with overfeeding, mice genetically engineered to lack PKR activity had less body fat, inflammation, and insulin resistance than mice with normal PKR after a number of weeks on a high-fat diet. Notably, when the mice were fed a regular diet, those lacking PKR activity were still more insulin sensitive than normal mice. These results suggest that PKR may coordinate both increased inflammation and metabolic malfunction in response to nutrient excess and other metabolic stresses. Understanding the regulation of PKR may also provide some insights into metabolic problems associated with viral infections. PKR may thus emerge as a therapeutic target in chronic metabolic diseases.

Nakamura T, Furuhashi M, Li P, et al. Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell 140: 338-348, 2010.

Discovery of a Pancreas-Specific Clock Associated with Diabetes: Scientists discovered that the pancreas has its own molecular clock, and that clock defects trigger onset of diabetes in a mouse model. In animals and humans, the circadian clock regulates many behaviors and bodily processes—including sleep/wake cycles, changes in blood pressure, and body temperature fluctuations—to harmonize these activities with daily, rhythmic changes in the environment, most notably day/night cycles. Metabolism is also rhythmically controlled, and disruption of these cycles is associated with type 2 diabetes. Researchers examined whether the circadian clock specifically played a role in regulating insulin release from pancreatic islets in response to glucose. The scientists found that, in a mouse model, the islets had their own molecular clock, distinct from the primary circadian clock that resides in the brain. When the researchers generally disrupted the circadian clock in mice, the animals had impaired tolerance to glucose, reduced insulin secretion, and smaller islets, suggesting that the pancreatic clock directly regulates insulin production. When the clock disruption was limited to the pancreas alone, the animals had elevated blood glucose levels and impaired insulin secretion in response to glucose, resulting in the animals developing diabetes. The research has identified a pancreas-specific clock—distinct from the body's overall circadian clock—that plays an important role in regulating metabolism. Targeting the proteins involved in regulating this clock is a possible strategy for treating diabetes.

Marcheva B, Ramsey KM, Buhr ED, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 466: 627-631, 2010.

TREATING SEVERE INSULIN RESISTANCE

Drug Combination Neutralizes Severe Insulin Resistance in a Rare Disease: A combination of therapeutic drugs has been found to reverse the devastating effects of "type B" insulin resistance. When the body becomes impaired in its ability to use insulin to regulate glucose uptake by cells, a variety of metabolic diseases can arise. One relatively rare but serious, often fatal, disease is type B insulin resistance. In this autoimmune condition, the body launches a misguided immune attack against insulin's protein partner, known as the insulin receptor, which resides on the surface of cells and effects the action of insulin. In people with type B insulin resistance, the body inappropriately produces antibodies that prevent interaction of insulin with its receptor, blocking insulin's ability to communicate with target cells. The resulting insulin

signal interference leads to blood glucose imbalance and numerous negative physiological effects, such as weight loss, excess testosterone production, and unusual skin discoloration (known as acanthosis nigricans). While the condition can affect anyone, type B insulin resistance occurs predominantly in African American women. Previously, therapeutic strategies to restore the body's ability to regulate glucose in patients with type B insulin resistance had only been modestly successful.

Recently, scientists in NIDDK's Intramural Research Program, in collaboration with other researchers, identified a combination of drugs that was remarkably effective in reversing the physiological effects of insulin resistance. This drug combination was designed to remove existing circulating antibodies, as well as to prevent the production of new antibodies, and included steroids and other immunosuppressive drugs, as well as a drug called rituximab, which causes the depletion of antibody-producing cells. Due to the rarity of this disease, only a few patients were treated in this study. However, the results were clear: all seven people with type B insulin resistance who received treatment were in remission, generally within 1 year. Importantly, there were minimal side effects from the treatment regimen. These findings not only establish a promising new treatment strategy for those suffering from type B insulin resistance, but also provide a framework for designing effective therapies for other autoimmune disorders.

Malek R, Chong AY, Lupsa BC, et al. Treatment of type B insulin resistance: a novel approach to reduce insulin receptor autoantibodies. J Clin Endocrinol Metab 95: 3641-3647, 2010.

CYSTIC FIBROSIS RESEARCH

Potential Avenues for Treating Cystic Fibrosis—Partially Restoring the Function of One Protein by Inhibiting the Function of Others: New research suggests it may one day be possible to treat many people with cystic fibrosis (CF) by inhibiting an enzyme called histone deacetylase 7 (HDAC7) and/or by interfering with the cell's protein "quality control" machinery. Most people with CF have a version of the CF gene that generates a misfolded form of CFTR, the most functionally significant CF protein. Because it is not folded correctly, this variant of CFTR does not reach its normal location at the cell surface, where it is needed to transport salts and keep the airways of the lungs properly hydrated. Researchers recently found that inhibiting the action of the HDAC7 protein allows some of the variant CFTR to fold so that it can reach the cell surface and function. These experiments were performed in cultured human lung cells, so it is not yet clear whether a similar approach can safely be taken to treat patients with CF. However, one of the compounds used in this study to inhibit HDAC7 already has been approved by the U.S. Food and Drug Administration for treatment of cutaneous T cell lymphoma, a form of cancer. It is not certain precisely how HDAC7 inhibition affects CFTR, but it is likely to be an indirect effect, possibly involving several other genes and proteins. Further research is needed to address this question. These results suggest that inhibiting HDAC proteins may be a viable approach for treating some people with CF, and possibly for treating people with other diseases related to protein folding problems.

Although strategies, such as inhibiting HDAC7, that increase the amount of CFTR reaching the cell surface are a promising approach to treatment, they are potentially hampered by the cell's protein quality control machinery, which both prevents abnormal proteins from reaching the cell surface and removes them from the surface if they do arrive—even if the abnormal proteins are partly functional or could be made functional with therapeutics. New research has helped clarify the set of proteins that are involved in recognizing and removing abnormal or misfolded CFTR from the cell surface, and targeting it to be degraded within the cell. Working with cultured human cells expressing the same misfolded CFTR variant referred to above, researchers used a molecular tool called small interfering RNAs (siRNAs) selectively to eliminate specific proteins involved in the cell's quality control process. By interfering with one of these proteins at a time, they were able to identify those that are integral to removing misfolded CFTR from the cell surface. Thus, it may one day be possible to increase the amount of functioning CFTR reaching the surface of the cell through the use of an inhibitor of HDAC7 or a related approach, while at the same time, inhibiting the cell's quality control machinery may increase the time that CFTR spends on the cell surface, where it functions as an ion transporter.

Hutt DM, Herman D, Rodrigues APC, et al. Reduced histone deacetylase 7 activity restores function to misfolded CFTR in cystic fibrosis. Nat Chem Biol 6: 25-33, 2010.

Okiyoneda T, Barrière H, Bagdány M, et al. Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science 329: 805-810, 2010.

Ferreting Out the Complex Biology of Cystic Fibrosis: Researchers have recently developed a new animal model of CF that may help answer important questions about the biology of the disease and advance the development and testing of new candidate CF therapies. Although chronic, damaging lung infections are the most frequent cause of death for people with CF, the disease also has a serious impact on the pancreas, liver, intestine, gallbladder, sweat glands, and male reproductive tract. Digestive consequences of the disease in the intestine and pancreas, for example, are of enormous significance for children with the disease because they severely interfere with the ability of the body to absorb key nutrients and fuel proper growth and development. Mouse and pig models of CF have helped clarify what goes wrong in the many organ systems affected by the disease, but their utility has been limited by differences between these animals and humans in organs and tissues and the effects of the disease. The ferret respiratory system is markedly more like humans' than is the airway of mice. In particular, the distribution of the CFTR protein is quite similar in the ferret and human airways.

For this reason, researchers generated a model of CF by eliminating the ferret *CFTR* gene in test animals, and examined the physiological consequences. They found that the effect on the ferret respiratory tract was much like what is seen in humans with CF, but the effect on the ferret digestive tract was more severe than is seen in people with this disease. To facilitate study of other aspects of the disease, the researchers found two ways of getting around the severe digestive complications of ferret CF: using medications that help alleviate these digestive consequences, and creating another line of animals in which CFTR is functional in the intestinal cells but not in other organs. Both approaches showed promise in yielding animals that may be of great potential value in terms of understanding the effect of CF on human organ systems, in particular the respiratory tract, and for helping to develop and test potential new therapeutic approaches to treating the disease.

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Dr. Muneesh Tewari and Dr. Martin T. Zanni: NIDDK-Supported Scientists Receive Presidential Award

On November 5, 2010, President Barack Obama recognized 85 U.S. scientists, including two supported by NIDDK, with the Presidential Early Career Award for Scientists and Engineers (PECASE; *www.whitehouse.gov/administration/eop/ostp/pressroom/11052010*).

PECASE is the most prestigious award given in the U.S. to scientists at the outset of their independent research careers. The 2009 recipients were honored at a White House ceremony with the President in December 2010.

PECASE is awarded annually to scientists and engineers who, while early in their research careers, have demonstrated the pursuit of innovative research and outstanding scientific leadership. Among the 2009 recipients are Muneesh Tewari, M.D., Ph.D. and Martin T. Zanni, Ph.D., both NIDDK extramural grantees.

In addition to Drs. Tewari and Zanni, 18 other NIH-supported scientists received the award for their research achievements. The NIH has now funded 173 PECASE recipients since the award's inception in 1996. A list of NIH scientists who have received this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

Investigations into a Potentially New Type of Hormone

Muneesh Tewari, M.D., Ph.D.

Dr. Tewari, an oncologist and cancer researcher at the Fred Hutchinson Cancer Research Center in Seattle, received a 2009 PECASE award for his innovative work on the release of RNA from cancer cells that may lead to early cancer detection with the potential for novel therapies. The conventional norms of the mammalian endocrine system do not generally consider RNA molecules as a class of hormones. Hormones are small molecules that are secreted into the blood and affect the function of distant tissues and organs. Dr. Tewari points to several facts to propose that RNA molecules may also function as hormones in mammals. First, RNA molecules have been demonstrated to function as hormones in plants. Second, in some animal species (*e.g.*, worms and flies) RNA has been shown to spread from one site to another. And third, one class of RNAs, known as microRNAs, are abundantly present in the blood of healthy people, and specific microRNAs accumulate in states such as cancer, diabetes, and other diseases. To lay the foundation of whether microRNAs can act as a type of hormone in mammals, Dr. Tewari is studying microRNAs secreted into the blood by cancer cells to determine whether they are taken up by and influence distant organs and tissues. Establishing that RNA molecules in the blood can act as hormones could lead to better methods of diagnosing and treating a variety of human diseases.

Developing Cutting-Edge Technologies To Study Health and Disease

Martin T. Zanni, Ph.D.

Dr. Zanni, the Meloche-Bascom Professor of Chemistry at the University of Wisconsin-Madison, received a 2009 PECASE award for his research developing novel spectroscopic methodologies to study the molecular mechanisms by which biomolecules cause disease. He specializes in the development of two-dimensional infrared (2D-IR) spectroscopy and its application to problems in biophysics and human health. He has broken new ground in understanding infectious diseases with a novel

discovery about the structure of the influenza virus's M2 protein, a major target of anti-influenza drugs. Dr. Zanni is currently using 2D-IR spectroscopy to uncover key details about amyloid toxicity with implications for the treatment of type 2 diabetes. A feature of type 2 diabetes is the presence of amyloid fibers in the pancreas. These fibers are composed of the human islet amyloid polypeptide (hIAPP), and many *in vitro* and *in vivo* studies have linked them to the disease. However, the mechanism by which hIAPP promotes the death of the insulin-producing cells is not understood. A growing body of evidence points to special molecular species of hIAPP interacting with the cell membrane as the cause of cell death rather than the fibers themselves. Using 2D-IR spectroscopy, Dr. Zanni is characterizing these molecular species and their interaction with the cell membrane to better understand their contribution to the development of type 2 diabetes.

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What's Old Is New Again: Targeting Inflammation To Treat Diabetes

Researchers have recently made an important clinical advance that has its origins in surprising observations about type 2 diabetes that began to accumulate well over a century ago. At that time, insulin had not yet been discovered, and there was no effective way to treat any form of diabetes. Indeed, aspirin had not yet been invented, although earlier forms of the drug—called salicylates—were known to reduce the pain, fever, and swelling of inflammation. In reports published in 1876 and 1901, clinicians found that high-dose salicylates partly alleviated diabetes as measured by the earliest known biomarker of the disease—glucose in the urine. Unfortunately, the approach was impractical, because high doses of salicylates have significant side effects and, in particular, are damaging to the stomach. Thus, for many decades the result remained a puzzle to researchers, to the extent that it was remembered at all. Why would anti-inflammatory medications like salicylates relieve diabetes when neither pain, nor any of the other hallmarks of inflammation known since antiquity—fever, swelling, and redness—are intrinsic to diabetes?

Inflammation is essentially an "SOS" signal sent by cells called macrophages to other components of the immune system. Macrophages send this signal in response to injury or infection, and gradually turn the signal off as the wound or infection heals. By the middle of the 20th century, scientists were beginning to piece together some of the molecular details of the inflammatory process. For example, the serum concentrations of a variety of proteins known as "acute phase proteins" were found to rise or fall in conjunction with inflammation. The first clue to understanding the surprising efficacy of salicylates came in the 1950s, when some of these acute phase proteins were found to be elevated in diabetes. At the time, the significance of this finding was not widely appreciated.

The connection between diabetes and inflammation did not begin to come into sharper focus until pioneering NIDDK-funded work in the 1990s found that an acute phase protein called TNF-alpha was produced in adipose (fat) tissues of obese mice. More provocatively, the researchers found that producing excess TNF-alpha in non-obese mice could induce insulin resistance, a condition that can lead to type 2 diabetes and that is also a hallmark of the disease. The researchers also found that deleting the TNF-alpha gene, or the gene of another protein that is required for its activity, actually protected mice from type 2 diabetes. These results suggested an answer to a question that had vexed the field for a long time—what is it about excess adipose tissue that promotes diabetes? The data raised the possibility that the immune system may play a role in insulin resistance, even in the absence of infection.

Indeed, NIDDK-supported researchers found that macrophages accumulate in the adipose tissue of obese rodents, as well as humans. But what is it about obesity that attracts macrophages to an uninjured, uninfected part of the body? The answer has to do with a group of proteins called Toll-like receptors (TLRs), which recognize foreign material in the body, such as molecules on the outside of invading bacteria, and in response trigger a powerful inflammatory signal. NIDDK-supported researchers found, in 2001, that one of these proteins, TLR4, which is produced both by macrophages and fat cells, is activated not only by bacteria, but also by high levels of free fatty acids, an important form

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of unstored fat in the body. Researchers found that mice lacking TLR4 are protected from insulin resistance caused by a high-fat diet, showing that signaling through TLR4 plays a key role in the central pathological process that leads to type 2 diabetes. Thus, it is now believed that the presence of high levels of free fatty acids in adipose tissue triggers TLR4 on fat cells to induce production of inflammatory signals, which draws macrophages to the tissue. Macrophage-produced TLR4 also responds to the fatty acids, ramping up the inflammatory process.

A parallel line of inquiry was clarifying the role of salicylates. These medicines, it turns out, can impinge on the inflammatory process in two distinct ways. First, they can inhibit the activity of an enzyme known as cyclooxygenase, which has a key role in inducing many of the classical symptoms of inflammation, including fever and pain. Second, they can interrupt an enzyme called I-kappa-B kinase beta (IKK-beta), which has a key role in transmitting the inflammatory signal stimulated by TNF-alpha, and which turns out to be relevant to diabetes. NIDDK-supported research showed that inhibiting IKK-beta can reverse insulin resistance in rodent models of type 2 diabetes, and that stimulating IKK-beta can trigger insulin resistance. These results help explain how salicylates might counteract type 2 diabetes. Additionally, IKK-beta is less sensitive to aspirin and other salicylates than is cyclooxygenase, a finding that may help explain why aspirin and related pain relievers, when given in moderate doses, do not alleviate diabetes. Indeed, as suggested by the clinical data on salicylates from the 19th century, high-dose salicylates were found to reverse insulin resistance in obese mice.

This basic research set the stage for a modern re-test of salicylates for the treatment of type 2 diabetes. In 2002, NIDDK-supported researchers reported the metabolic effect of high-dose aspirin on seven people with type 2 diabetes. The treatment significantly improved insulin sensitivity, and lowered levels of blood glucose, cholesterol, and other fats, without affecting body weight. As with the salicylates tested more than a century earlier, the significant side effects of high-dose aspirin, which can include gastrointestinal ulcers and bleeding, especially in the stomach, make it unsuitable as a long-term therapy for diabetes. However, the result demonstrated that IKK-beta inhibition might be a pharmacologically effective approach for treating type 2 diabetes, if a method could be found to do so safely.

It turned out that a form of salicylate was already known which seemed to have suitable properties. Salsalate, a pain medication used for decades as a treatment for rheumatoid arthritis, is notable for lacking many of the high-dose side effects of aspirin, and is available as an inexpensive, generic prescription drug. Small, preliminary trials of salsalate indicated that it may be effective as a treatment for type 2 diabetes. The NIDDK established the Targeting INflammation Using SALsalate in Type 2 Diabetes (TINSAL-T2D) clinical study to more rigorously test the approach. In the trial, 108 participants who had recently been diagnosed with type 2 diabetes were randomly assigned to receive either placebo or one of three different doses of salsalate, for 14 weeks. Researchers tracked changes in participants' hemoglobin A1c (HbA1c) levels—used to track blood glucose control—as well as other metabolic measures, and also monitored for side effects.

As reported in 2010, those participants receiving any of the three doses of salsalate experienced a significant reduction in HbA1c level compared to those in the placebo group, indicating that salsalate was effective in improving blood glucose control. Those who took salsalate also saw improvements in their blood levels of triglycerides (a type of fat), suggesting the salsalate might help lower their risk of cardiovascular disease, which has also been linked to inflammation. Although there were

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not many side effects, one was that salsalate was associated with an increase in the concentration of a protein called albumin in the urine. Urine albumin is a marker of kidney disease, which is an important complication of diabetes, so this observation could indicate a problem with salsalate as a type 2 diabetes treatment. In contrast, another marker of kidney function, cystatin C, did not change in any of the groups over the course of the trial, and thus it is unclear whether or not salsalate might harm kidney function. This question will be examined carefully in future tests. In addition, participants receiving salsalate were more likely than those receiving placebo to experience mild episodes of low blood glucose. This side effect was only observed in patients who were also taking a diabetes medication known as sulfonylurea, suggesting the two drugs may not be suitable for use in tandem.

The blood glucose level finding is also an indication that salsalate is lowering blood glucose, because diabetes is essentially the state of chronically elevated blood glucose.

Based on these encouraging results, an expanded study of safety and efficacy is now being conducted in a larger number of patients, with a longer duration of treatment. Designated "TINSAL-T2D-Stage II," the trial is recruiting participants for a 48-week treatment course with salsalate or placebo. If the approach is successful, this research could offer a new treatment option for diabetes using an inexpensive medication that has been found to be safe and effective over decades of use in patients with arthritis. And the use of anti-inflammatory agents for diabetes, first tested more than 130 years ago, may soon reach fruition as a therapeutic approach.

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Gestational Diabetes and the Health of Generations

Answers about the causes of human obesity and type 2 diabetes may lie not only with genetic and environmental factors, but also with factors encountered while in the womb. With the hope of understanding and potentially preventing chronic disease, researchers have been studying the impact of maternal diabetes, particularly gestational diabetes mellitus (GDM)—a form of diabetes similar to type 2 diabetes, but unique to pregnancy—on the health of women and their offspring. From these studies, we now know that GDM is not simply a temporary condition with potentially acute consequences during late pregnancy and delivery. Rather, diabetes during pregnancy appears to increase long-term metabolic health risks for both mother and child. Moreover, results from a major clinical study of maternal blood glucose levels and pregnancy outcomes have led clinicians to reconsider current criteria for diagnosing and treating GDM. These discoveries are providing new opportunities to improve the health of women and their families.

A form of diabetes that is first diagnosed during pregnancy, GDM is estimated to affect about 7 percent of all U.S. pregnancies—about 200,000 each year.[1] While it usually resolves after delivery, research has shown that women who have been diagnosed with GDM are at significantly increased risk of having it again during future pregnancies and/or getting diabetes, primarily type 2 diabetes, later in life. GDM also increases the risk of complications for mother and child during pregnancy and delivery. While the cause of GDM is not fully known, it is thought that hormonal changes during pregnancy contribute to its development.

As early as 1952, researchers recognized that a pregnant woman's metabolic health could have an impact on the developing fetus. A Danish scientist, Dr. Jørgen Pedersen, proposed that elevated blood glucose levels in the mother lead to elevated glucose levels in the infant, who then responds by increasing insulin production, which promotes storage of energy as fat—even to excess. In subsequent years, this hypothesis was further refined as more was learned about the contributors to an over-nutritive intrauterine environment and its effects on a developing fetus, a concept known as "fuel-mediated teratogenesis."

In addition to the immediate consequences of maternal diabetes on offspring, scientists were curious about the impact that these early exposures in the intrauterine environment might have long-term. Scientists in NIDDK's Intramural Research Program were among the first to shed light on these questions. Working with the Pima Indians in Phoenix, Arizona, a population having one of the highest rates of obesity and type 2 diabetes in the world, NIDDK researchers have been able to learn a great deal about the genetic and environmental factors influencing development of these conditions. In 1983, NIDDK researchers published the results of a study of children born to Pima Indian women with and without diabetes during pregnancy. These included both women who developed GDM and women who already had type 2 diabetes prior to pregnancy. The results clearly linked maternal diabetes during pregnancy with higher rates of obesity in offspring during their childhood and teen years. Subsequently, in the late 1980s, the research team found that offspring of Pima Indian women who had diabetes during pregnancy were much more likely to develop type 2 diabetes as young adults compared to children of women who did not have diabetes during pregnancy. Another key study was published in 2000, in which NIDDK researchers examined Pima Indian mothers with type 2 diabetes who had multiple pregnancies—some before they developed the disease, and some after. The children born from a diabetic pregnancy had a higher chance of being obese or developing diabetes than those—from the

same mother—born from pregnancies before the mother developed type 2 diabetes. This study allowed the scientists to zero in on the effect of the intrauterine environment *per se*—because the babies were born to the same mother, genetic factors were not playing a role. Importantly, these and other studies in the Pima Indians enabled researchers to determine that the intrauterine environment exerts an effect on the development of obesity and diabetes in youth that is distinct from genetic and environmental contributors.

Do the findings in the Pima Indian population hold true in more diverse populations in the U.S.? Answers have emerged from the SEARCH for Diabetes in Youth (SEARCH) study. SEARCH is examining the prevalence and incidence of diabetes in racially and ethnically diverse children and youth, as well as elucidating factors that contribute to development of diabetes before the age of 20. SEARCH researchers have found that maternal diabetes appears to accelerate onset of type 2 diabetes in youth. Among study participants with type 2 diabetes, those whose mothers had diabetes during pregnancy were diagnosed at a younger age than those whose mothers were diabetes free. A related study using SEARCH data found that youth with type 2 diabetes were much more likely to have been exposed to diabetes or obesity while *in utero* than youth without diabetes. These results indicate a universal effect of intrauterine exposure that operates, in addition to factors such as genetics and race/ethnicity, to increase lifetime risk for developing type 2 diabetes.

Spurred by these studies in people, researchers have also studied the impact of maternal diabetes in animals. Together, the studies in people and animals have led to the current model that "diabetes begets diabetes." A key aspect of this model is the threat of a vicious cycle, in which daughters of diabetic pregnancies develop diabetes themselves prior to or at the time of pregnancy, perpetuating the impact of diabetes on offspring via the intrauterine environment. The urgency of these findings is underscored by findings in the last several years among the Pima Indians, demonstrating an increased incidence of diabetes complications at an earlier age among people diagnosed with type 2 diabetes in youth. As there are an increasing number of people developing diabetes and obesity at younger ages, it will be important to try and break this vicious cycle by preventing or mitigating the effects of diabetes and obesity during child-bearing years and pregnancy.

The criteria for GDM were originally established based on risk to the mother of subsequently developing type 2 diabetes. Therefore, an important question has been whether GDM—or even hyperglycemia at levels below traditional GDM criteria—has adverse pregnancy outcomes for mother and child. The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, led by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development with co-support from NIDDK, sought to determine whether blood glucose levels that are lower than those used to diagnose GDM have acute consequences on pregnancy outcomes. By examining blood glucose levels and pregnancy outcomes in over 23,000 women around the world, the HAPO study demonstrated that there is a continuum of risk according to glycemia at levels below those currently used to diagnose GDM. In the study, the higher a pregnant woman's blood glucose levels following an oral glucose tolerance test, the more likely she was to deliver a high-birthweight baby, and the more likely the baby was to have signs of hyperinsulinemia (excessive levels of circulating insulin in the blood). Elevated blood glucose also raised the risk for extremely serious problems for the mother and child, such as preeclampsia in the mother and shoulder dystocia for the newborn during birth. Most significantly, there was no clear threshold level at which these outcomes began—that is, there is apparently no "safe" level of hyperglycemia. As a result of the HAPO study and other clinical studies, an international panel has recommended new criteria

for diagnosing GDM. These criteria would potentially increase the burden of gestational diabetes from 7 to nearly 18 percent of pregnancies, and have now been adopted by the American Diabetes Association.

Scientists are still striving to understand the biological underpinnings of the long-term health effects of diabetes and obesity during pregnancy. Studies in humans, as well as in animal models, are under way to try to understand the mechanisms by which the intrauterine environment determines disease later in life. Research indicates that obesity and hyperglycemia during pregnancy are associated with epigenetic changes, which are changes in the regulation of gene activity and expression (whether genes are turned on or off) that are not dependent on the sequence of the DNA. While scientists work to understand the mechanisms underlying "metabolic imprinting," continued research could help determine how best to protect the growing fetus from an adverse metabolic milieu, and break the vicious cycle of trans-generational transmission of metabolic disease.

While many questions remain, the work of numerous researchers over several decades revealed that diabetes during pregnancy has long-term effects on the metabolic health of mothers and their children.

The National Diabetes Education Program (NDEP), which is co-led by NIDDK and the Centers for Disease Control and Prevention (CDC), is a platform for disseminating evidence-based information about prevention and treatment of diabetes and its complications. In partnership with the NIH Office of Research on Women's Health, NDEP is translating what is known about GDM, through a campaign called "It's Never Too Early to Prevent Diabetes." This campaign is reaching out to women and their health care providers to raise awareness that women with a history of GDM are at increased risk of diabetes, and their children are at increased risk of type 2 diabetes and obesity. It also imparts a hopeful message, based on the results of the NIDDK-led Diabetes Prevention Program clinical trial, namely that type 2 diabetes can be prevented or delayed in women with a history of GDM through an intensive lifestyle intervention of diet and exercise or through use of the diabetes drug metformin. Continued research on this front will help break the vicious cycle and help to avert the serious health problems of diabetes and obesity in future generations.

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[1] *American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 27: S88-S90, 2004.*

Chronic Disease and the Regulation of Inflammation

Dr. Christopher K. Glass

Dr. Christopher K. Glass holds joint appointments in the Department of Medicine and in the Department of Cellular and Molecular Medicine at the University of California, San Diego (UCSD). Dr. Glass received his Bachelors degree in Biophysics from the University of California, Berkeley and his M.D. and Ph.D. degrees from UCSD. Following internship and residency training in Internal Medicine at Harvard Medical School's Brigham and Women's Hospital, Dr. Glass returned to UCSD for clinical and research fellowships in Endocrinology and Metabolism. Dr. Glass' laboratory currently investigates roles of certain protein factors in regulating the development and function of an important group of immune system cells, work which he presented at the May 2010 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.

Inflammation and Chronic Disease

Dr. Glass introduced inflammation—and how it is normally regulated—in the context of its beneficial effects in fighting infection. The immune system defends the body against infection by identifying microbial invaders, and destroying them. White blood cells called macrophages migrate out of the circulatory system into tissues throughout the body to initiate this process by triggering inflammation at sites of infection or injury. Inflammation is often associated with heat, pain, redness, or swelling, and serves as a physiological siren to recruit other immune cells to the site of infection. The inflammatory response that follows is part of the way in which the body fights and recovers from the infection. When the wound heals, or the infection has been cleared, these symptoms disappear, as macrophages complete the process by deactivating the inflammatory response.

Inflammation is not always good for the body, however. In the last several years, researchers have discovered that some chronic, unhealthy conditions such as obesity also trigger a low-level inflammatory response in the absence of classic signs of inflammation, such as fever or pain. For example, macrophages accumulate in adipose (fat) tissue of obese individuals, triggering an inflammatory "defense" mechanism. Unlike the situation in a resolved infection, inflammation associated with obesity persists. This prolonged inflammation can lead to abnormal cell function and is thus thought to contribute to several common, chronic diseases, including heart disease and type 2 diabetes. A macrophage protein called Toll-like receptor 4 (TLR4), which recognizes an important class of bacteria and can initiate inflammation, also recognizes a variety of substances that are non-infectious, such as free fatty acids in the body, and may thus contribute to atherosclerosis, which is associated with heart disease; insulin resistance, which can lead to type 2 diabetes and is also a hallmark of this disease; and other chronic diseases, such as arthritis.

Dr. Glass discussed research from his laboratory that is unraveling at the molecular level how macrophages regulate inflammation and, in particular, attenuate the process when it is no longer needed, such as when an infection has resolved. His studies are also shedding light on the causes of many chronic diseases, and helping to identify potential avenues of intervention to treat or prevent them.

An early clue about the counter-regulation of the inflammatory process from the 1940s helped establish the theoretical underpinnings of the Glass laboratory's research. Researchers Edward Kendall

and Philip Hensch discovered that the steroid hormone cortisone could help relieve the inflammation and pain of rheumatoid arthritis. Unfortunately, long-term use of cortisone can result in significant undesirable side effects. Cortisone, it was later found, works by activating a type of protein called a "nuclear receptor."

The Nuclear Receptor Signaling Atlas (NURSA)

Nuclear receptors are proteins that help certain hormones—including cortisone, estrogen and testosterone, and many other substances, such as vitamin D—exert some of their effects. Typically, when one of these hormones (or other substances) enters a cell, it binds to its "receptor," and the hormone-receptor complex then interacts both with specific DNA sites and with other proteins to turn some genes in the cell's nucleus "on" and others "off." That is, the hormone-receptor complex triggers the cells to begin more actively utilizing genes that had been either dormant or only on at a relatively low level, or to stop utilizing a gene that was active prior to the signal. "Expression" level refers effectively to the extent that the cellular machinery is actively making the product or products of a particular gene.

With support from NIDDK, the National Heart, Lung, and Blood Institute, and the National Institute of Environmental Health Sciences, the Nuclear Receptor Signaling Atlas (NURSA) project is cataloging the diverse and extremely important physiological effects of nuclear receptor proteins, the hormones and other signals that stimulate them, and their many physiological effects, as well as their roles in disease.

NURSA research has helped document the tissues and cell types in which the 48 human nuclear receptors are found, and have shown that 28 of them are present in macrophages. Many undergo significant changes in their own expression levels in response to inflammatory stimuli. Each responds to its own set of chemical or hormonal signals to turn on and off different groups of genes. Dr. Glass' laboratory is studying the impact of macrophage nuclear receptors on inflammation.

Nuclear Receptors and Inflammation

Dr. Glass and his team found that stimulating macrophages with a molecule from pathogenic bacteria that is a powerful trigger of TLR4 boosted expression of over 500 different genes, many of them linked to the inflammatory response. The team then selectively stimulated three different nuclear hormone receptors, and explored the impact each one had on the array of genes that had been turned on by the bacterial trigger molecule. Each nuclear receptor reduced expression of hundreds of the inflammatory genes, but none turned off all of them. Some of the genes were turned off by just one of the receptors, some by two, and some by any one of the three. However, many remained unaffected by any of the three nuclear receptors examined in the experiment. Importantly, in addition to turning off various genes, each nuclear receptor also turns on a set of genes. Thus, it became clear that any set of stimuli is likely to yield a unique, complex, and finely tuned physiological response.

The potential of the nuclear receptors to tamp down an inflammatory response makes them particularly attractive as drug targets for treatment of pain, prevention of transplanted organ rejection, or prevention of atherosclerosis or type 2 diabetes. However, the complex cellular reaction from stimulating any individual receptor can make for undesirable side effects, as illustrated by the result of cortisone treatment for rheumatoid arthritis. Thus, researchers aim to understand how nuclear receptors do what they do, so as to harness them more selectively for therapeutic benefits while minimizing side effects.

An important example of a nuclear receptor that has been selectively targeted for this sort of strategy is PPAR-gamma, which is involved not only in control of inflammation by macrophages, but also in fat cell

development and, importantly, in regulating the levels of glucose in the body through its effects on fat, liver, and muscle cells. A class of diabetes pharmaceuticals known as thiazolidinediones, including rosiglitazone (sold as Avandia®) and pioglitazone (sold as Actos®), are PPAR-gamma stimulants that have been shown to be effective at helping people with type 2 diabetes control their blood glucose. Unfortunately, their use is also associated with increases in body fat, swelling associated with water retention in tissues (edema), and reduced bone formation. Rosiglitazone also has been linked, in some studies, to a higher risk of cardiovascular events, such as heart attack and stroke.

The Many Facets of the Nuclear Receptor PPAR-gamma in Health and Disease

In experiments in mice, Dr. Glass and his colleagues selectively eliminated PPAR-gamma in macrophages, leaving it intact in other cells, and found that the result was exaggerated inflammatory gene expression, and a loss of impact of the thiazolidinedione drugs on inflammation. This indicates that PPAR-gamma in macrophages has a much more profound effect on inflammation than PPAR-gamma in other cell types. Dr. Glass and his coworkers then looked at the impact of thiazolidinediones on glucose levels in two different groups of mice with type 2 diabetes: one group had PPAR-gamma in their macrophages, and the other did not. Both groups of mice had normally functioning PPAR-gamma in other tissues. The researchers found that the medications helped lower blood glucose in both groups of mice, but were more effective in the mice that had the nuclear receptor in their macrophages. This indicates that macrophage PPAR-gamma has a significant impact on glucose control—a surprise, because it was thought that liver, muscle, and fat cells are the sites where PPAR-gamma has its greatest impact on glucose levels.

They next examined a set of naturally occurring mutations in the PPAR-gamma gene that are known to affect human health. Most such mutations result in lipodystrophy, a disease in which fat cells are not normally distributed in the body. In PPAR-gamma-associated lipodystrophy, there is essentially no fat under skin in the extremities, but there is extra fat in the central parts of the body. Many of the mutations also cause type 2 diabetes and/or heart disease. In further analysis, Dr. Glass noted that all of the PPAR-gamma mutations that cause lipodystrophy also reduced PPAR-gamma's capacity to boost expression of other genes in the presence of receptor stimulants like rosiglitazone. One such mutation, however, reduced the capacity of PPAR-gamma to stimulate gene expression while leaving intact the ability of this nuclear receptor to reduce inflammation; notably, this mutation did not cause type 2 diabetes. This observation suggests that it may be possible to separate the functions of PPAR-gamma to reduce inflammation without stimulating other pathways through gene expression, and thus to treat type 2 diabetes and other inflammatory diseases, but avoid the side effects of thiazolidinedione treatment. A major goal of Dr. Glass' laboratory is to achieve exactly that.

Conclusion

Going forward, the Glass laboratory is also zeroing in on the specific cellular functions of PPAR-gamma and other nuclear receptors. New tools are allowing them to determine the many proteins these receptors are in physical contact with in the cell, and helping determine exactly what role they play in various specific tissues within a living animal. These avenues of inquiry will help complete the complex picture of what these important proteins do in health and disease.

PATIENT PROFILE

Nilia Olsen

Participating in TEDDY To Identify What Triggers Type 1 Diabetes in Children

Nilia Olsen

Four-year-old Nilia Olsen has no idea she's participating in a study that has determined she has an elevated risk for developing type 1 diabetes. She just knows that, every 3 months, she goes to the doctor's office to have her blood drawn, and, of all things, "she loves it," says her mom, Sonya.

"She likes the different colors on the tops of the vials," says Sonya, referring to the collection vials for blood samples. "Her favorite color is pink, so she likes to fill that one up first."

Nilia is one of over 8,000 children participating in The Environmental Determinants of Diabetes in the Young study, otherwise known as TEDDY. TEDDY is led by NIDDK and supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*.

The international study's long-term goal is to try to identify infectious agents, dietary factors, or other environmental agents, including psychosocial factors, that trigger type 1 diabetes in genetically susceptible individuals or protect against the disease.

But such details don't concern Nilia right now. She's a typical little girl who attends pre-school, likes to dress up—as well as dig for worms. "She's high energy," her mother laughs.

About the TEDDY Study

Researchers have discovered that children who develop type 1 diabetes have certain kinds of "high-risk" genes. Analyzing DNA from Nilia's blood shortly after she was born indicated that she was genetically at high risk to develop the disease. Researchers also know that some children with high-risk genes develop type 1 diabetes, while others don't. This has led them to think that something in the environment "triggers" or causes a child with high-risk genes to actually get type 1 diabetes. The purpose of TEDDY, therefore, is to try to identify the environmental triggers that cause children to get the disease. TEDDY has enrolled genetically susceptible newborns into the study from two populations: those with a sibling or parent with type 1 diabetes and those from the general population with no family history of the disease. Nilia falls into the general population group because she has no family history of type 1 diabetes.

Like Nilia, the other children in this study, all of whom were identified within 3 months of their birth as being at high genetic risk for developing type 1 diabetes, will be followed until age 15. During that time, information will be collected about their diets,

PATIENT PROFILE

illnesses, allergies, and other life experiences. Blood samples will be collected every 3 months and stool samples will be collected monthly for the first 4 years. After 4 years, these samples will be collected every 6 months until the children turn 15 years old. Parents are also asked to fill out questionnaires at regular intervals, and to record events, such as illnesses, in the child's "TEDDY Book."

From these numerous samples and other information collected about the children, researchers hope to identify a factor or factors that lead some genetically predisposed children to develop type 1 diabetes while others do not. This information is critically important for identifying strategies for disease prevention. For example, if a virus were found to trigger type 1 diabetes, a vaccine could possibly be developed. If a dietary factor were found to be causative, then changes to children's diets could be made.

It is only through the dedicated efforts of families, such as the Olsen family, that the TEDDY study could be conducted. Responsibilities such as making regular doctor's visits for blood draws, taking monthly stool samples for 4 years, and keeping detailed notebooks with information about their child's health is no small task. It is clear that TEDDY families are dedicated to the study and its goals. This commitment can reap major rewards if an environmental trigger is discovered, which could pave the way toward being able to prevent type 1 diabetes and help future generations of children.

One Family's Experience with the TEDDY Study

The day after Nilia was born, Sonya was asked if her daughter's blood could be sampled for a study to see if the child had an elevated risk for type 1 diabetes. Although there is no history of type 1 diabetes in the family, Sonya was aware that nearly all of the women on her father's side have type 2 diabetes (formerly called adult-onset diabetes). Sonya immediately agreed and enrolled Nilia into TEDDY when researchers found that Nilia carried high-risk genes for type 1 diabetes.

"I don't have diabetes, and neither does my husband, Thomas," says Sonya. Thomas is in the military and was deployed to Iraq on his second tour of duty in October 2009. Beyond her daughter's increased genetic risk for type 1 diabetes, Sonya says that an additional motivation for her to enroll Nilia into TEDDY was "knowing that type 2 diabetes runs on my father's side of the family." Both forms of diabetes can lead to serious health complications.

"I would strongly encourage other families to participate in clinical trials like TEDDY," says Sonya. "Knowing that our daughter is in a trial like TEDDY gives us a great deal of peace of mind."

At the time this profile was written, it was 4 years since Nilia's blood was originally sampled, and although it was discovered that she has an elevated risk for developing type 1 diabetes, fortunately she remains diabetes free. Sonya says that knowing Nilia has an elevated risk "was something that as a family we knew we would take in stride." Not only have they taken it in stride, they have remained dedicated to contributing to an important research study that can lead to new ways to prevent type 1 diabetes. According to TEDDY staff, Sonya has always gone the extra mile to do everything she could for TEDDY research, including participating in optional fun events designed to build community among local TEDDY families. However, the Olsen family lived in Augusta, Georgia, when Nilia first entered the study. Since then, the family has been transferred by the military to Alabama. "The study staff has been very flexible," says Sonya. "We do everything through the mail. Every time Nilia's blood and stool samples get tested, they mail us the results. It's great. The continuity provides us with a sense of peace."

As with all other study participants, the Olsen family was provided a calendar, "and we record whenever

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Nilia goes to the doctor," says Sonya. "We write down when she's sick and what kind of medication she may be taking. We record if she goes to the hospital." But so far, according to Sonya, Nilia has not had any hospitalizations or health issues related to diabetes. As for filling out the calendar and other paperwork related to the study, "It's not difficult, at all," says Sonya. "It just takes a few minutes to record."

Nilia may be a big reason the family is able to cope so well. At age 4, she is proving to be a real trooper when it comes to participating in the study. "Whenever she goes to the bathroom, she asks if she needs to poop in the cup," Sonya says, with a slight laugh. "And she's really good at having her blood drawn. She never cries about it and never has." In fact, Nilia is so good about having her blood drawn that she is featured in a video about blood draws for TEDDY that has been distributed to other TEDDY sites.

"I would strongly encourage other families to participate in clinical trials like TEDDY," says Sonya. "Knowing that our daughter is in a trial like TEDDY gives us a great deal of peace of mind."

Nilia's father, Thomas, calls home once a week from Iraq and asks how Nilia is doing. "It pleases me to be able to tell him she's doing well."

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Joan Pasquesi

HAPO Study Brings Results—and Comfort

Joan Pasquesi and her three children

When Joan Pasquesi, a nurse at Northwestern Memorial Hospital, in Illinois, learned that a study related to gestational diabetes was going to be conducted at her research center, she jumped at the opportunity to participate—and for good reason.

"I've never had any health issues related to diabetes," says Joan, "but my father passed away at age 63 from complications of type 2 diabetes."

At the time the study started, Joan was pregnant with her first child. Knowing that diabetes has a strong genetic link, and that many of her father's cousins and aunts also had type 2 diabetes, Joan saw the study as a step she could take to help protect her health and that of her future children. She now has two sons and a daughter ranging in age from 4 to 10 years, all of whom are diabetes free. Still, Joan remains watchful, aware that her family history means that she and her children are likely at greater risk of developing diabetes. Says Joan, "Knowing what my father went through forces me to be more vigilant."

The study that Joan enrolled in was a noninvasive, observational study called the Hyperglycemia and Adverse Pregnancy Outcomes, or HAPO, study. While it is known that overt diabetes places women and their babies at higher risk for complications during pregnancy and delivery, there has been longstanding debate as to whether such risks apply to gestational diabetes. Spearheaded by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), with co-support from NIDDK, the purpose of HAPO was to clarify whether even mildly elevated maternal blood glucose (sugar) levels—*i.e.*, lower than the levels currently used to diagnose diabetes—increase the risk of adverse outcomes for pregnant women and their babies.

Says Joan, "Knowing what my father went through forces me to be more vigilant."

About Hyperglycemia and Gestational Diabetes

Gestational diabetes mellitus (GDM) is a form of diabetes that occurs during pregnancy. Like other forms of the disease, it is characterized by high blood glucose levels and can have serious consequences. Left untreated or uncontrolled, GDM can result in babies being born very large—9 pounds and over—and with extra fat, which can make delivery difficult and riskier for both mother and child.

While the exact cause of GDM is not known, it is thought that hormonal changes that occur during pregnancy are a major contributor. These changes impair the action of insulin in body tissues, causing a pregnant woman's blood glucose levels to rise—a condition known as hyperglycemia. In approximately 7 percent of pregnancies, levels are high enough, by current criteria, to indicate the presence of diabetes. While any pregnant woman may be at risk for GDM,

strong risk factors include obesity and a family history of type 2 diabetes.

For most women, GDM resolves after the baby is born, but leaves them at greater risk for the disease during subsequent pregnancies. GDM also increases the offspring's risk for obesity, and leaves both mother and child at increased risk for diabetes for the rest of their lives—risks that, given her father's experience with type 2 diabetes, did not escape Joan.

The Need To Stay Vigilant

"My father was one of those guys who never went to see a doctor," says Joan. "He felt he had no need. At age 44 he was feeling fine. He had never been admitted into a hospital nor missed a day of work in his life," she adds. "He was one of those guys whose attitude was 'if it ain't broke…'."

Despite her father's reluctance, Joan's mother convinced him to have a routine check-up.

"I was only 9 years old at the time, but I can remember it as if it happened yesterday," says Joan. "Shortly after his first general check-up in years, we received an urgent call from my father's physician, who told him to get into the hospital immediately." The doctor told Joan's family that her father's glucose levels were extremely high and "out of control," she continues. "He was at immediate risk for suffering a stroke or heart attack. We were all scared to death, including my father."

As it turned out, Joan's father lived to age 63. But, before he died, both his legs had to be amputated, he suffered multiple heart attacks and strokes, and he was on kidney dialysis—all of which are recognized as serious complications of type 2 diabetes. Joan wasn't about to risk a similar fate for herself or her children.

The HAPO Study

Joan's enrollment in the HAPO study placed her among a very diverse group of more than 25,000 other women who also were having their blood glucose levels examined during pregnancy in the study's 15 centers located in nine countries around the world. In addition, Joan was selected to take part in a training video to help ensure that the study would be carried out uniformly at all 15 centers.

According to Boyd E. Metzger, M.D., at the Northwestern University Feinberg School of Medicine, who led the study, training was critical to HAPO. "From drawing blood, to sending it to the lab, shipping it, and entering the data forms, all had to be consistent and conform to protocol," he says.

As for the study itself, Joan says, for the added comfort and security it provided her during her pregnancy, it required minimal time or effort. "The study was well run by knowledgeable coordinators and nurses," she adds. "To have these people as additional resources, and to know that my personal physician coordinated with them during my pregnancy was reassuring."

At about 28 weeks into their pregnancies, HAPO study participants were given an oral glucose tolerance test. This test is used to determine if a person has higher than normal levels of glucose in their blood 1 to 3 hours after drinking a standardized sugary drink. This test not only provided information about blood glucose levels below the threshold for diabetes, but also helped HAPO researchers to find women whose blood glucose levels were already high enough to meet a predefined threshold for treatment. In Joan's case, they were not. Had they been, she, like any participant in the study who met or exceeded the threshold, would have been made aware of the results so that her physician could begin treatment. Otherwise, it was a "blinded" study, meaning that unless the testing revealed overt diabetes requiring treatment, or a different test conducted later in pregnancy revealed an emerging problem with hyperglycemia, the HAPO participants, as well as the HAPO study staff (except laboratory

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personnel), remained unaware of their glucose levels during the study.

To see whether elevated maternal blood glucose levels below levels diagnostic for diabetes are associated with adverse pregnancy outcomes, the HAPO researchers also collected information about each participant's pregnancy and delivery. For example, they looked at whether the mother needed a cesarean section, or whether a baby experienced certain types of injury during vaginal delivery. Also, at the time of their birth, blood was taken from the baby's umbilical cord and tested for elevated insulin levels. A heel-stick blood glucose test was done approximately 2 hours after birth to detect hypoglycemia. High fetal insulin levels and a low newborn blood sugar level are two potential effects of maternal hyperglycemia on the baby. A study coordinator came within 24 hours of the birth to measure the baby's weight and skin fold thickness, an estimate of fat tissue. "A month later, they followed up to ask how my baby and I were doing," says Joan. "It was all very comforting and well coordinated." Ultimately, blood glucose data and pregnancy and birth outcomes from over 23,300 women were included in the HAPO study.

Results of the Study

The HAPO study demonstrated that even modest elevation of maternal blood glucose below the level that is diagnostic of diabetes is associated with adverse birth and pregnancy outcomes—meaning that the optimal levels of blood glucose during pregnancy are lower than previously appreciated.

The results of the HAPO study were "very convincing," says Dr. Metzger. The higher a pregnant woman's blood glucose levels, the more likely she is to deliver a high birth-weight baby, and the more likely the baby is to have signs of hyperinsulinemia, a condition in which there are excessive levels of circulating insulin in the blood.

HAPO demonstrated that even modest elevation of maternal blood glucose levels is associated with adverse birth and pregnancy outcomes—meaning that the optimal levels of blood glucose during pregnancy are lower than previously appreciated.

The study results also suggest that elevated blood glucose is associated with a greater risk of problems in the mother, such as preeclampsia, a potentially serious condition that only occurs during pregnancy in which high blood pressure and protein in the urine develop after the 20th week (late 2nd or 3rd trimester) of pregnancy. Usually the high blood pressure, protein in the urine, and other effects of preeclampsia go away completely within 6 weeks after delivery. However, sometimes the high blood pressure will get worse in the first several days after delivery.

As a result of the HAPO study, an international panel of researchers and physicians was assembled to examine its findings and the findings of related studies. The panel's recommendations for developing a new strategy for diagnosing GDM, which includes lowering the diagnostic threshold for GDM based on hyperglycemia testing, have been adopted by the American Diabetes Association.

"Given all I know about the deadly complications of diabetes as a result of my father's experience, this study was a no-brainer for me. I'd participate again, if I could."

The HAPO study results are also providing a framework for research questions focused on improving health outcomes for pregnant women and their offspring. Researchers believe, for example, that an observed increase in the rate of

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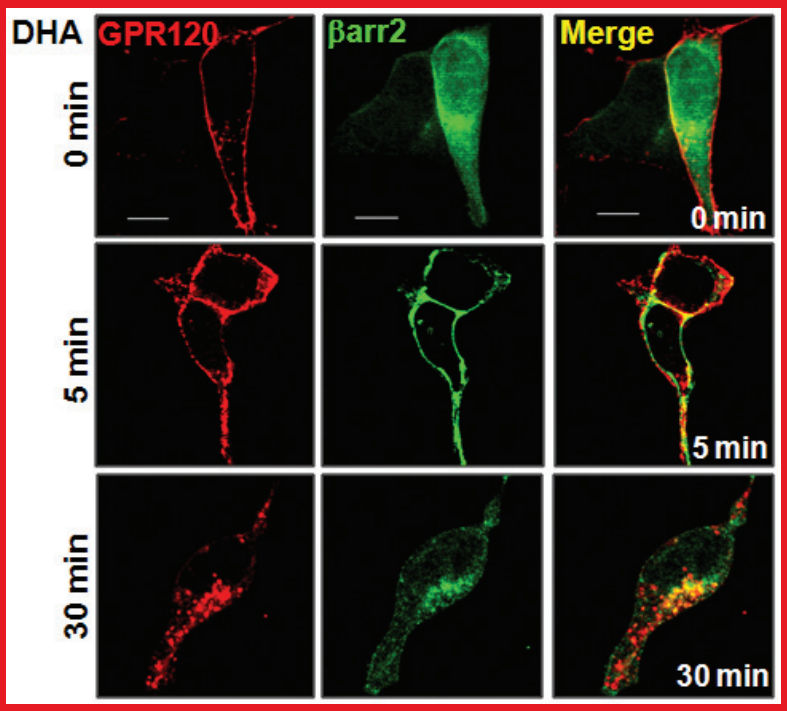
GDM globally is driven in large part by increased rates of overweight and obesity in women of childbearing age. These factors may also contribute to elevated glucose levels like those seen in women in the HAPO study. One approach to minimizing risk for all these women and their babies would be to monitor and minimize excessive weight gain during pregnancy—questions that may be pursued through research. Based on the HAPO results, researchers can also now explore questions such as the level of risk of future type 2 diabetes associated with GDM diagnosed by the new criteria or other long-term health risks in the mother and child.

As for Joan, "Given all I know about the deadly complications of diabetes as a result of my father's experience, this study was a no-brainer for me," she says. "I'd participate again, if I could."



Researchers have visualized the first steps in how fish oil exerts anti-inflammatory effects. This image shows how two cellular proteins, GPR120 (stained red) and beta-arrestin-2 (stained green), interact following exposure to DHA, an omega-3 fatty acid found in fish oil. When the two proteins are in the same place, a yellow signal results ("merge" column). Before DHA treatment, the two proteins are separate—GPR120 is at the cell surface, as shown by the red outline around the cell in the top row, while beta-arrestin-2 is inside the cell, seen as green within this cell. The addition of DHA causes beta-arrestin-2 to move to the cell surface, where GPR120 resides, observed as red, green, and yellow outlines of the cells in the middle row. Later, both proteins move together into the cell's interior, as observed by the red, green, and yellow throughout the cell in the bottom row. As described in an advance in this chapter, this apparent interaction between GPR120 and beta-arrestin-2 following DHA treatment is suspected to block the production of inflammatory molecules that promote insulin resistance in obesity. This research provides important insights into how omega-3 fatty acids may exert their anti-inflammatory effects and could potentially pave the way to new therapeutic options to treat inflammation and insulin resistance.

Images provided by Dr. Jerrold M. Olefsky and reprinted from Cell, 142, Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, and Olefsky JM, GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects, 687-698, copyright 2010, with permission from Elsevier.

Obesity

Obesity has risen to epidemic levels in the U.S. Individuals who are obese may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within NIDDK's mission.

Approximately one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] Nearly 17 percent of children and teens ages 2 through 19 are also obese, and thus at increased risk for developing serious diseases both during their youth and later in adulthood.[3] Obesity disproportionately affects people from certain racial and ethnic groups and those who are socio-economically disadvantaged.

The high prevalence of obesity in the U.S. 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. 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 of a variety of approaches for preventing and treating obesity include behavioral and environmental approaches in families, schools, and other community settings; medical interventions; and combinations of these strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight will spark new ideas for intervention approaches. To help bring research results to health care providers and the public, the Institute also sponsors education and information programs.

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. Recently, the Task Force developed an updated *Strategic Plan for NIH Obesity Research*, with extensive external input from researchers across the country, professional and other health-focused organizations, and others. The new *Strategic Plan* will reflect the exciting opportunities that have emerged from research progress in the years since NIH developed its first *Strategic Plan* on this major public health challenge. The *Strategic Plan* will be published in early 2011 and will be available on the NIH Web site.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. These represent examples of NIDDK's broad spectrum of research efforts toward reducing the burden of obesity so that people can look forward to healthier lives.

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[1] Statistics Related to Overweight and Obesity. http://win.niddk.nih.gov/statistics/index.htm

[2] Flegal KM, et al: JAMA 303: 235-241, 2010.

[3] Ogden CL, et al: JAMA 303: 242-249, 2010. 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).

RISK FACTORS FOR PREMATURE DEATH

Obesity and Related Conditions in Childhood Predict Premature Death: A new study has found that obesity, glucose intolerance, and elevated blood pressure during childhood and adolescence are associated with increased rates of early death. Adult obesity is known to be associated with cardiovascular disease, type 2 diabetes, and premature mortality, but similar relationships with pediatric overweight and obesity have not been clearly shown. By identifying risk factors in adolescence, researchers and clinicians can more effectively prevent and manage chronic diseases before they become more problematic later in life. In this decades-long study, researchers monitored the health of Pima and Tohono O'odham Indians from the Gila River Indian Community in Arizona, a population with the highest known prevalence of type 2 diabetes in the world.

The study participants included almost 5,000 non-diabetic American Indian children and youth. The researchers monitored their health through adulthood, and found several risk factors in children that were associated with risk for early death—before the age of 55—from a variety of causes (not including external causes such as accidents). The 25 percent of participants in the study who had the highest body mass indices (BMI, a measure of weight relative to height) during childhood or adolescence were more than twice as likely to die early than the 25 percent with the lowest BMIs. Similarly, based on results from glucose tolerance testing, the quarter of the group with the highest blood glucose levels early in life, reflecting underlying insulin resistance, were 73 percent more likely to die prematurely than were those with the lowest blood glucose levels. Hypertension was also a pediatric risk factor—the premature death rate among participants with elevated blood pressure was found to be 57 percent higher than those with lower blood pressure. In contrast, cholesterol levels in this group were not linked to extra risk for premature death. Although this study focused on an American Indian population, the rate of obesity in many other ethnic and racial groups has risen dramatically in the past 3 decades. Thus, further study will be important to determine whether youth of other backgrounds are similarly endangered by high BMI, insulin resistance, or high blood pressure. The high prevalence of childhood obesity is of great public health concern, and these findings further underscore the need to prevent and treat obesity early in life.

Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, and Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. N Engl J Med 362: 485-493, 2010.

REGULATORS OF METABOLISM IN THE BRAIN

Exploring How the Brain Controls Energy-Burning Fat: Scientists have discovered that the activity of brown adipose tissue (BAT), a form of fat that burns calories to generate heat, decreases in adult animals that had been administered monosodium glutamate (MSG) as newborns. Unlike "white fat" which stores fats as an energy reserve, BAT actually uses calories to generate heat that keeps animals warm. Recently, much attention has been given to how the central nervous system is able to control the body's metabolism, and the link between the brain and fat tissue function. In particular, a part of the brain called the arcuate nucleus (Arc) is thought to be important in regulating appetite and influencing calorie burning (energy expenditure) to fuel activity and generate body heat. Previous studies in mice demonstrated that lesions in the Arc produced by neonatal administration of MSG resulted in obesity and decreased BAT activity. However, the precise reasons for why damage to the Arc results in effects on BAT function remain unclear.

Researchers performed a series of experiments in Siberian hamsters to further explore the connection between impaired BAT heat production and Arc damage from MSG exposure, as a way to increase understanding of the regulation of this fat-burning tissue. At 3 months of age, hamsters that were treated with MSG injections as newborns had significantly increased body mass compared to ones that were never given MSG treatment ("control" animals). Additionally, the MSG-exposed hamsters had increased white fat mass compared to the controls. To measure BAT function, both the MSG-treated and control hamsters were exposed to the cold, and the temperature of their BAT was directly measured. Initially, the BAT in both groups of hamsters was able to produce similar temperatures, but the MSG-treated

hamsters were unable to maintain BAT temperature after 2 hours. By 18 hours, the BAT temperature in the MSG-treated animals had decreased significantly compared to the control animals. The inability of the BAT to generate heat in MSG-treated hamsters was not due to an intrinsic defect in the tissue itself since it was able to maintain a constant temperature in a manner similar to the controls when stimulated by a hormone (rather than by cold exposure). The researchers therefore examined the animals' brains to find factors that could possibly account for the disrupted BAT function. Similar to previous studies, neonatal MSG exposure resulted in profound destruction in certain regions of the Arc. Surprisingly, the remaining Arc neurons in the MSG-treated animals appeared intact and similar to the control animals. Additionally, other parts of the brain believed to influence BAT function were also unaffected by MSG treatment. These results indicate that the impaired BAT function is apparently not due to MSG-induced central nervous system damage, but does seem to be caused by a factor that is extrinsic to the BAT. Further research to increase understanding of the factors influencing BAT energy expenditure may help the development of new ways of regulating metabolism, controlling body weight, and combating obesity.

Leitner C and Bartness TJ. Acute brown adipose tissue temperature response to cold in monosodium glutamate-treated Siberian hamsters. Brain Res 1292: 38-51, 2009.

Role for Brain in Cholesterol Regulation: A new study in rodent models suggests that the brain plays a role in regulating cholesterol levels in the blood. Essential for cellular function and activities, cholesterol consumed in the diet or synthesized by the liver circulates throughout the body in special complexes with proteins, referred to as HDL (good) cholesterol and LDL (bad) cholesterol. While it is not fully understood how cholesterol levels are regulated, it is well known that too much LDL cholesterol raises risk for atherosclerosis and cardiovascular disease, whereas increased levels of HDL cholesterol have the opposite effect. Research has shown that many metabolic activities are regulated centrally by a part of the brain, called the hypothalamus, in response to molecular cues received from the gut, pancreas, fat, and other tissues. In this study, researchers asked whether the melanocortin system—a neural circuit in the hypothalamus that is key to regulating body fat, blood pressure, and glucose metabolism—might also regulate cholesterol levels. Two gut hormones, ghrelin and GLP-1, have opposite effects on this neural circuit. Ghrelin inhibits the melanocortin system, increasing appetite and food intake, while GLP-1, a "satiety hormone," stimulates it, reducing food intake and promoting energy expenditure (calorie burning). Through a series of experiments in rodents, the researchers discovered that administering ghrelin or a similarly acting compound into the brain caused total cholesterol levels to rise, while injecting GLP-1 caused them to fall. Further, mice genetically engineered to lack a key component of the melanocortin system also had higher cholesterol levels than their normal counterparts. Intriguingly, the study results indicate that the majority of the increase in total cholesterol caused by blocking the melanocortin system was due to reduced reuptake of HDL cholesterol by liver cells—the body's method for clearing and recycling cholesterol. While the relevance of these results from rodents to regulation of cholesterol in humans has yet to be determined, the study has uncovered yet another possible role for the brain in regulating metabolic activities.

Perez-Tilve D, Hofmann SM, Basford J, et al. Melanocortin signaling in the CNS directly regulates circulating cholesterol. Nat Neurosci 13: 877-882, 2010.

Wiring of Nerve Cell Connections in the Brain Could Affect Obesity: A new research study that was conducted in rodents indicates that resistance to obesity may be conferred by sets of brain cell connections. While a majority of people are susceptible to obesity, a fraction of the population appears resistant to weight gain. The molecular basis for this disparity is not understood. In this report, scientists studied rats and mice to gain greater insight into the role that neuron (nerve cell) wiring in the brain plays in obesity. A series of brain circuits called the central melanocortin system is thought to play a key role in regulating the body's ability to feel hunger or satiety and respond accordingly.

To explore this idea, researchers took advantage of a well-studied laboratory rat population that can be divided into two groups that respond differently to a high-fat diet. The "DIO" rat group fed a high-fat diet is vulnerable to diet-induced obesity, whereas

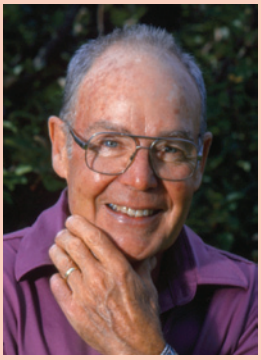
the "DR" rat group is resistant. When the two groups were shifted from a standard diet to a high-fat diet, the DIO rats gained significantly more weight than did the DR rats. The scientists discovered several differences between the brains of obesity-prone and obesity-resistant rats. For example, feeding the rats a high-fat diet affected the number of connections on certain brain cells, called POMC neurons. These neurons are part of the melanocortin system, which is located in the hypothalamus area of the brain, and respond to hormonal signals. In the DIO rats, POMC connections were lost. In contrast, the DR rats' POMC connections increased. The researchers also examined another aspect of neurons in the animals. Typically, neurons are wrapped by cells called astrocytes, which provide protection, support, and a physical environment necessary for proper brain function. These cells are also thought to contribute to the blood-brain barrier, which helps to restrict microorganisms and certain chemicals from entering the brain. The scientists discovered that when the rats were fed a high-fat diet, a greater number of astrocytes were associated with POMC neurons of DIO rats compared with DR rats. The scientists theorized that this increase in the number of astrocytes, a process called gliosis, may limit the ability of satiety hormones to regulate POMC neurons in DIO rats by preventing the development of new neuronal connections, leading to poorly controlled feeding behavior. The scientists also conducted "standard diet" versus "high-fat diet" experiments in mice. Like the rats, two groups of mice that responded differently to a high-fat diet exhibited the same correlation of increased gliosis with weight gain.

Researchers have shown in this study that diet-induced weight gain in rats and mice that are vulnerable to obesity is related to the gliosis of POMC neurons that are located in the hypothalamus. A possible cause of weight gain is that gliosis insulates these neurons from hormonal signals that communicate satiety. If this relationship is also found in people, it would indicate that altered brain wiring connections that are caused by gliosis in response to dietary shifts may play a role in human obesity.

Horvath TL, Sarman B, García-Cáceres C, et al. Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. Proc Natl Acad Sci USA 107: 14875-14880, 2010.

2010 ALBERT LASKER BASIC MEDICAL RESEARCH AWARD

The Discovery of Leptin




Drs. Douglas Coleman (left) and Jeffrey Friedman
Photos courtesy of The Lasker Foundation

Drs. Douglas L. Coleman (Jackson Laboratory) and Jeffrey M. Friedman (Rockefeller University) received the 2010 Albert Lasker Basic Medical Research Award for their discovery of leptin, a hormone that regulates appetite and body weight. This discovery ignited an explosion of research into the molecular circuitry that controls food intake, metabolism, and body weight, and spotlighted the role of adipose (fat) tissue in regulating metabolism. It changed perceptions about the causes of obesity.

In the 1960s and 1970s, Dr. Coleman—a former long-time NIDDK grantee—studied two different types of mice that shared the characteristic of being strikingly obese. These mice had defects in what was referred to as the *diabetes* (*db*) gene or in the *obese* (*ob*) gene, which are separate genes on separate chromosomes. At that time, however, he did not have the genes in hand—the technology for such a feat was still decades away. Based on previous research, Dr. Coleman reasoned that the genetic defects in these mice altered a blood-borne satiety factor that contributed to the severity of obesity and diabetes.

To test this hypothesis, Dr. Coleman conducted experiments with different mice to see whether blood from one mouse might affect the appetite or weight of another. These experiments showed that the blood of mice with the *ob* defect did not have this proposed satiety factor, a result suggesting that the mice were obese because they didn't produce this factor. By contrast, the experiments showed that mice with the *db* defect made too much of the proposed satiety factor, but were nonetheless obese because they could not respond to it. These results, however, faced skepticism from the scientific community because at that time, obesity was considered to be simply a behavioral, not a physiological, problem.

It was not until 2 decades later that Dr. Coleman's proposed satiety factor was identified, and it was determined that obesity can have a physiologic basis. In 1994, with support from NIDDK, Dr. Friedman and his colleagues were the first research team to identify the *ob* gene in mice and in humans. They found that this gene encoded a protein hormone—the factor that Dr. Coleman had earlier inferred must exist. Dr. Friedman called this hormone "leptin," after the Greek word for "thin." Subsequently, researchers also showed that the *db* gene made a protein that interacts with leptin, called the leptin receptor, which is essential for leptin to have its effects. Even more importantly, Dr. Friedman's research showed that leptin was made by adipose tissue and signaled to the brain, where the receptor was located, to regulate appetite and energy expenditure. In rare cases, people suffering from extreme obesity may have a genetic defect that causes them to not make enough leptin to properly regulate their food intake. Researchers have shown that injections of leptin can dramatically reduce appetite and promote weight loss in these individuals.

The discovery of leptin by Drs. Coleman and Friedman dramatically altered the landscape of obesity research. This discovery revealed that adipose tissue more than passively stores fat; it is, in fact, an endocrine organ. Research fueled by this discovery has uncovered a number of other substances that, like leptin, are secreted by fat cells and converge in the brain to control food intake and body weight. In addition, this discovery changed the prevailing view that obesity was a behavioral problem involving solely a lack of willpower. On the contrary, the discovery of leptin highlighted the previously unappreciated molecular component of obesity. As a result, obesity is now addressed as a multi-faceted problem involving a myriad of genetic, molecular, environmental, and behavioral factors that regulate appetite, metabolism, and body weight.

IMMUNE CELL REGULATOR OF WEIGHT LOSS AND INSULIN SENSITIVITY

Signaling Protein Provides New Clues about the Benefits of Omega-3 Fatty Acids: A new study has uncovered a mechanism for the anti-inflammatory effects of omega-3 fatty acids, and shown that they can also enhance insulin sensitivity in mice. Chronic inflammation in fat tissue contributes to the development of insulin resistance seen in obesity. Omega-3 fatty acids, found in fish oil and other foods, reduce inflammation, but the mechanisms by which they exert this effect are not well understood.

Researchers examined the possible role of a signaling protein found in large numbers at the surface of macrophages—cells of the immune system that promote inflammation—and mature fat cells. Through experiments in mouse macrophages, they discovered that cells with the protein, called GPR120, could resist becoming pro-inflammatory if treated with the omega-3 fatty acid, docosahexaenoic acid (DHA). However, when they experimentally reduced the amount of GPR120 produced by the macrophages, the cells no longer responded to the DHA treatment—indicating that GPR120 is important for mediating the anti-inflammatory effects of this omega-3 fatty acid. Other experiments yielded clues as to how GPR120 reduces inflammation, suggesting that DHA treatment mobilizes a third molecule (beta-arrestin-2) to interact with GPR120 and subsequently block the cell's production of inflammatory molecules—including those that can promote insulin resistance. In mouse fat cells, DHA treatment also had the salutary effect of enhancing sensitivity to insulin action in a GPR120-dependent fashion. Having thus far examined isolated mouse cells, the scientists next investigated the impact of GPR120 on inflammation and insulin sensitivity in living animals. To do this, they genetically engineered mice to lack the GPR120 protein and compared these mice to their normal counterparts. Not only were the mice lacking GPR120 innately more insulin resistant, but when both sets of mice were switched to a diet enriched with omega-3 fatty acids after nearly 4 months on a "regular" high-fat diet, mice lacking GPR120 showed no improvement, while the normal mice showed metabolic improvement and decreased inflammation. This experiment confirmed that GPR120 was critical for the anti-inflammatory effect of omega-3 fatty acids in mice, as had been shown in isolated mouse cells. These results provide important insights into how omega-3 fatty acids may exert their anti-inflammatory effects and could potentially pave the way to new therapeutic options to treat inflammation and insulin resistance.

Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 142: 687-698, 2010.

GUT MICROBES, ADIPOSITY, AND OBESITY

Intestinal Bacteria May Contribute to Developing Obesity and Diabetes: Scientists recently discovered that intestinal bacteria may have an important role in the development of metabolic syndrome, diabetes, and obesity. Metabolic syndrome is a constellation of disorders that increases the risk of developing diabetes and cardiovascular disease. Hallmarks include elevated blood glucose (sugar), insulin resistance, abnormal blood cholesterol, high blood pressure, fatty liver disease, and obesity—particularly excess abdominal fat. Bacteria that inhabit the digestive tract appear to influence metabolism by affecting the ability to extract energy from food. Furthermore, certain types of intestinal bacteria may play a role in developing obesity, type 2 diabetes, and other aspects of metabolic syndrome. The types of bacteria populating the gut may be determined in part by a protein called Toll-like receptor (TLR) 5. This protein is produced in abundance by cells in the intestinal lining, is important for recognizing microbes, and is part of the innate immune system that can respond to infectious bacteria. Mice lacking TLR5 develop intestinal infections and gain weight, leading scientists to believe that the protein may also influence metabolism, potentially by altering normal gut bacteria.

To further explore the role of intestinal bacteria and TLR5 in developing metabolic syndrome, scientists generated mice that do not produce TLR5 protein. These animals weighed about 20 percent more than normal mice by 20 weeks of age, consumed about 10 percent more food, and produced more body fat compared to their normal counterparts.

The TLR5-deficient mice also developed elevated cholesterol levels, increased blood pressure, and insulin resistance. When fed a high-fat diet for 8 weeks, both normal and TLR5-deficient mice gained weight, but unlike normal mice, TLR5-deficient animals developed type 2 diabetes and fatty livers. One possible explanation was that TLR5-deficient mice ate a greater quantity of high-fat food. When scientists restricted the amount of high-fat food so that TLR5-deficient mice and normal mice ate the same quantity, the TLR5-deficient animals did not become obese, but were insulin resistant. The investigators thought that the metabolic differences observed between the two strains of mice were due to changes in intestinal bacterial populations resulting from the loss of TLR5. Thus, the researchers compared the gut bacteria between the normal and TLR5-deficient mice and uncovered differences in levels of over 100 types of bacteria. To assess whether the changes in bacteria might be causing the metabolic symptoms, the scientists collected intestinal bacteria from TLR5-deficient mice and transplanted these into "germ-free" mice raised in a bacteria-free environment. Similar to TLR5-deficient mice, the mice who received the transplanted bacteria increased their food consumption, developed insulin resistance, and became obese.

This study demonstrates that bacteria in the gut may contribute to changes in appetite and metabolism. Excess calorie consumption along with the resulting obesity and development of type 2 diabetes could possibly be driven, at least in part, by alterations in intestinal bacteria populations due to biological pathways involving TLR5. Understanding how gut bacteria interact with the intestine could provide a means of modulating eating behavior, as well as preventing metabolic syndrome.

Vijay-Kumar M, Aitken JD, Carvalho FA, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 328: 228-231, 2010.

NEW INSIGHTS INTO FAT

Identification of a Key Mediator of Fat Cell Development: Scientists used a novel quantitative method to identify molecular regulators needed to form cells with the potential to become fatty tissues in mice and thereby discovered a critical component of the developmental process that generates fatty (adipose) tissues. Significant progress has been made in uncovering the factors and mechanisms that control the process by which mature fat cells (adipocytes) develop from cells with the potential to become adipocytes (preadipocytes) in response to molecular cues. Little is known, however, about how cells become or are maintained as preadipocytes. To identify preadipocyte regulators, the researchers generated new mouse cell lines and evaluated their capacity to form adipocytes. Creation of cell lines with strong propensity to develop into fat cells, as well as lines with little such capacity, allowed the scientists to use a new quantitative molecular tool they developed to identify regulators present at different levels in these different types of cells. They reasoned that factors important for directing cells to be preadipocytes would likely be present at much greater levels in precursors of fat cells compared to precursors of other types of cells or mature fat cells. One factor in particular, called Zfp423, was chosen for further characterization. As one way to test its role in preadipocytes, the scientists engineered high levels of Zfp423 into cells that would not ordinarily have the capacity to become fat cells and looked to see what happened. They found that increased levels of Zfp423 were able to drive these cells to become adipocytes.

The scientists also tested Zfp423's importance with another approach. Using molecular biology techniques, the scientists decreased levels of Zfp423 in preadipocytes and observed that these cells had reduced ability to become adipocytes and had lower levels of other molecular markers characteristic of adipocytes. They demonstrated that Zfp423 is an important regulator of one of these molecular markers—the *PPAR-gamma* gene, itself another regulator of fat cell development. The researchers also found that Zfp423 plays a key role in production of both major forms of fat in mice (brown fat and white fat). Thus, this important study described a new tool for identifying molecular regulators of cell development and revealed the important role of Zfp423 to the preadiopocyte state. Understanding the formation and properties of fat can help inform strategies to prevent or reduce obesity.

Gupta RK, Arany Z, Seale P, et al. Transcriptional control of preadipocyte determination by Zfp423. Nature 464: 619-623, 2010.

Transitioning from an Early-Stage to Mature Fat Cell: New research has revealed the existence of a critical, transient intermediate state in the formation of fat cells from precursor cells having the potential to become fat cells. Previous research identified molecules involved in the formation of fat, but little was known about how this process is initiated once a precursor cell receives a signal to transition to a mature fat cell. This new study identified a chemical "signature" in the proteins associated with DNA that appears after mouse fat precursors are induced to become mature fat cells. By determining the location of this signature throughout the genome, the scientists were able to identify two proteins—CEBP-beta and the glucocorticoid receptor—that are important in initiating this transition state. These two proteins appear to work together to translate the signal and turn on genes important in the maturation of fat cells, including the master regulator of fat formation, PPAR-gamma protein. The identification of this transition state and the molecular components involved reveals new potential targets and strategies to combat obesity.

Steger DJ, Grant GR, Schupp M, et al. Propagation of adipogenic signals through an epigenomic transition state. Genes Dev 24: 1035-1044, 2010.

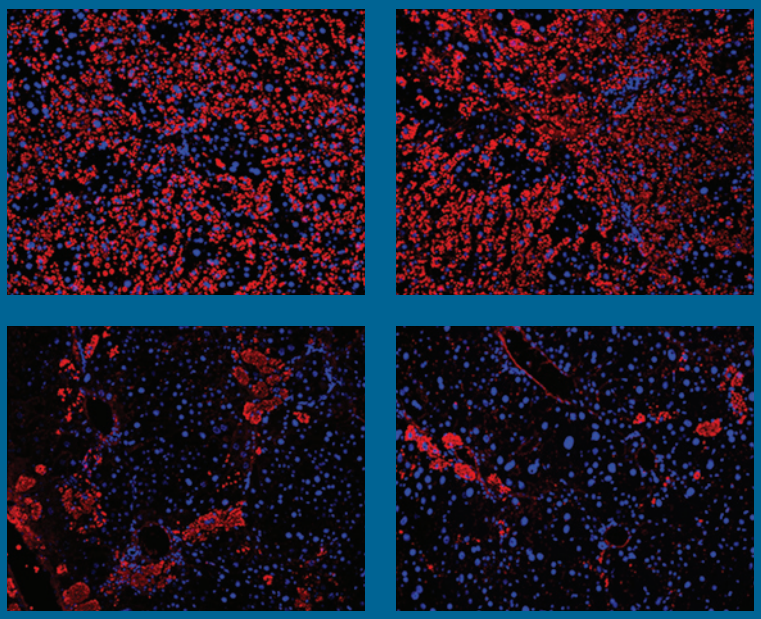
RESEARCH ON BARIATRIC SURGERY

Practice Might Not Make Perfect, But It Helps—Surgeons Who Perform More Bariatric Surgical Procedures Have Better Patient Outcomes: The Longitudinal Assessment of Bariatric Surgery (LABS)-1, a prospective observational study examining the 30-day adverse outcomes of bariatric surgery, has revealed that surgeons who perform bariatric surgery more frequently have significantly fewer patients who suffer from complications of this procedure. Obesity is a major public health concern that is associated with increased risk for type 2 diabetes, coronary heart disease, stroke, fatty liver disease, certain types of cancer, and other diseases. Bariatric surgical procedures modify the digestive tract to limit the amount of food that can enter the stomach, decrease absorption of nutrients, or both, and are used to treat extreme obesity. Bariatric surgery has consistently resulted in substantial and sustained weight loss for people with extreme obesity, and has been linked to remission of type 2 diabetes, decreases in cardiovascular risk factors, and reduced mortality over time. However, this operation is technically demanding, and patients undergoing the surgery are frequently at risk for serious complications, including death. One factor that can greatly influence complication rates is the experience of the surgeon performing the operation.

Researchers participating in LABS-1 examined the relationship between the surgeon's experience in performing a type of bariatric surgery called Roux-en-Y gastric bypass (RYGB) and the patients' short-term (30-day) outcomes. Thirty-one surgeons at 10 different centers who are a part of the LABS-1 collaborative participated in the study. From 3,410 initial bariatric surgical procedures performed during the study, the researchers analyzed outcome data with respect to whether the patients had any of several complications, including venous thrombosis, pulmonary embolism, reoperation, non-discharge at 30 days post-operation, repeat hospitalization within 30 days following initial discharge, or death. They also took into account health differences among the patients prior to surgery. After adjusting for individual patients' health characteristics, the investigators found that surgeons who had performed more bariatric surgeries were associated with lower rates of patients with postsurgical complications. The study concluded that for every 10 additional surgical procedures performed per year, the surgeons' rate of patient complications decreased by 10 percent.

These findings support the concept that the more experienced a surgeon is with bariatric surgical procedures, the lower is the risk of adverse post-operative outcomes. Bariatric surgery is an effective weight-loss procedure that is becoming increasingly popular as a treatment for extreme obesity. The safety of such surgery is a critical consideration, with risks examined in the context of long-term benefits. This study emphasizes that the experience of the surgeon performing the procedure can have a significant impact on patient outcome. This and other components of the LABS-1 study are building evidence about the risks and benefits of bariatric surgery, to help patients and health care providers make more informed decisions about undergoing this procedure.

Smith MD, Patterson E, Wahed AS, et al. Relationship between surgeon volume and adverse outcomes after RYGB in Longitudinal Assessment of Bariatric Surgery (LABS) study. Surg Obes Relat Dis 6: 118-125, 2010.



These sections of liver tissue, taken from a mouse model of liver damage associated with the disease alpha-1 antitrypsin deficiency, show reduced accumulation of the mutated alpha-1 antitrypsin protein (in red) in mice given the drug carbamazepine (lower two panels) compared to untreated mice (upper two panels). As described in this chapter, researchers have found that this drug, previously used for other conditions, shows promise in pre-clinical studies as a treatment for alpha-1 antitrypsin deficiency, a form of genetic liver disease that affects children as well as adults.

Images provided by Drs. Tunda Hidvegi and David H. Perlmutter from Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, Maurice N, Mukherjee A, Goldbach C, Watkins S, Michalopoulos G, and Perlmutter DH: An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science 329: 229-232, 2010. Reprinted with permission from AAAS.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the U.S. 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. In 2004, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare, collectively, they exact 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. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the U.S. and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive 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 may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. The continued discovery of predisposing genetic variations and potential autoimmune and microbial influences 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. Screening programs for colorectal cancer are aimed at reducing mortality through early detection, particularly in those individuals at higher risk.

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 transform into an intestinal type of cell, is associated with a heightened risk of esophageal cancer, the most rapidly rising cancer in the U.S. Gastroparesis is another functional bowel disorder that is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to

[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

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 results in damage to the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with host cells, and with nutrients ingested by their host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract, as well as other systems throughout the body, such as those with immune and metabolic functions.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis, and their complications. Acute pancreatitis is typically caused by gallstones, while common causes of the chronic form include inherited genetic factors and heavy alcohol use. In both forms, digestive enzymes attack the pancreas from within, causing inflammation and pain. Research has elucidated genetic 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, as well as distribution of nutrients such as fats. When the liver is functionally compromised by disease, this can have serious adverse effects on health and can sometimes lead 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) known as nonalcoholic steatohepatitis. In recent years, however, NAFLD has been increasingly diagnosed in children in the U.S. as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency, others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis 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 of critical importance to identify liver disease early, preserve liver function in people with liver disease, and develop new treatment options, including transplants performed with liver tissue from living donors.

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, 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, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss 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 alterations in nutrient metabolism, such as an inherited form of copper deficiency called Menkes

disease. NIDDK-supported research has enhanced knowledge of how this nutritional disorder and others develop, and how they can best be treated.

GENETICS OF INFLAMMATORY BOWEL DISEASES

Genetic Risk Factors Associated with Inflammatory Bowel Diseases in Children:

Researchers have identified five new genetic variations that predispose children to developing inflammatory bowel diseases (IBD). The two major forms of IBD—Crohn's disease and ulcerative colitis—are marked by chronic and destructive inflammation in the intestinal tract. While the precise causes of IBD are unknown, there is a strong genetic component that predisposes individuals to developing these diseases. Previously, nearly 50 genetic risk factors had been identified as associated with IBD. Many of these genetic factors involve components of the immune system that control intestinal inflammation. These factors, however, were typically identified in adults with IBD. Since some clinical aspects of IBD in children differ from those in adults, it was important to extend these studies to identify genetic risk factors associated with the development of IBD in pediatric populations.

An international team of researchers has carried out the largest genome-wide association (GWA) study to date for identifying genetic variants associated with IBD in children. In a GWA study, scientists scan thousands of genomes for genetic variants that are more common in individuals with a disease—such as a form of IBD—than in healthy individuals. In this study comparing children with IBD and healthy children, the researchers identified variants in five new regions of the genome that increase children's risk of developing IBD. One of the most prominent risk factors identified for Crohn's disease was found near the *IL27* gene. This gene codes for a protein that is involved in an immune response previously implicated in the pathogenesis of Crohn's disease. The researchers showed that colon cells from children with Crohn's disease had much lower levels of *IL27* gene activity than cells from healthy individuals, suggesting that the risk variant reduces the amount of protein made from the *IL27* gene. In addition to the five new genetic regions, the pediatric population also has many of the genetic risk factors previously identified in adults with Crohn's disease or ulcerative colitis. This implies that IBD involve similar biological pathways in adults and children, but that IBD in children may involve some distinct pathways as well. Defining the genetic variations that predispose individuals to developing IBD enables researchers to gain insight into the causes of these diseases and potentially develop strategies to help detect, treat, and prevent early-onset IBD in children.

Imielinski M, Baldassano RN, Griffiths A, et al. Common variants at five new loci associated with early-onset inflammatory bowel disease. Nat Genet 41: 1335-1340, 2009.

New Genetic Risk Factors Associated with Ulcerative Colitis: Researchers have identified new genetic risk factors for ulcerative colitis (UC), one of the major forms of IBD. UC causes inflammation and ulcers in the lining of the rectum and colon. Previous genetic analyses have suggested that UC and Crohn's disease—the other major form of IBD—share some but not all of the susceptibility genes that would predispose some individuals to develop these diseases. However, to date, far fewer genetic risk factors have been identified in people with UC as compared to Crohn's disease.

In an effort to identify additional genetic variations underlying UC, an international team of researchers, including investigators from the NIDDK IBD Genetics Consortium, carried out two new GWA studies comparing the genetic variation of individuals with UC to the genomes of healthy individuals. In addition, they analyzed these new data sets in conjunction with data from a previously reported study to increase the sample size and also the likelihood of identifying UC-associated genetic variants. From this combined analysis, the scientists identified at least 30 distinct risk factors for ulcerative colitis, about half of which were previously unknown. In addition, this analysis revealed genetic variants that Crohn's disease and UC share in common; several variants previously known only for their association with Crohn's disease were found to be associated with UC. To help determine the biological implications of the genetic associations, the researchers examined the activity of candidate genes located near five genomic regions containing UC-associated variants. The activity of these genes in different tissues and cell types highlighted the potential importance of

several cellular functions in the pathogenesis of UC, including the integrity of the cells lining the intestine, which normally form a barrier between the intestine's contents and the rest of the body; the response to cellular "stress," such as the presence of improperly shaped proteins within a cell; and the induction and resolution of inflammation. This combined analysis enhances the understanding of disease mechanisms underlying UC, and how they overlap or are distinct from Crohn's disease. However, researchers estimate that these findings explain only a fraction of the genetic risk for UC. Therefore, further research is needed to identify additional susceptibility genes that improve our knowledge of UC and inform future strategies to manage this disease.

McGovern DPB, Gardet A, Törkvist L, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet 42: 332-337, 2010.

Variants in Gene Cluster Associated with Chronic Inflammatory Diseases: An international network of researchers has uncovered a set of genetic variants in a region of the human genome that is associated with chronic inflammatory diseases, such as IBD. This genomic region, called the major histocompatibility complex (MHC), contains a cluster of genes that play an important role in the immune system. A subset of MHC genes—referred to as *HLA* genes in humans—encodes a set of proteins that present foreign molecules to the immune system to generate a response that fights infection. While this response is designed to be protective, the *HLA* genes have been implicated in a number of autoimmune diseases, in which the body mounts an inappropriate immune response against itself. The intrinsic genetic complexity and variability in the MHC region, however, has limited the ability of researchers to identify the precise genetic variants associated with autoimmune disease.

Researchers collaborating through a network spanning the U.S. and Europe have now identified genetic variants within the MHC that are unique to and shared across multiple chronic inflammatory diseases with autoimmune features, including Crohn's disease and ulcerative colitis—the two major forms of IBD—and several other diseases. By scanning the MHC region of thousands of DNA samples from healthy individuals and patients with these diseases, the researchers were able to uncover the most common genetic variants associated with these diseases. For Crohn's disease and ulcerative colitis, the genetic scan identified a version of the *HLA-DRB1* gene as being the primary genetic factor within the MHC region associated with disease susceptibility. In addition, the large number of samples used in this study allowed the researchers to identify additional, secondary genetic variants within the MHC that may also contribute to the risk for these diseases. These results suggest that a complex pattern of genetic variants across the entire MHC region is associated with the risk of developing IBD and other chronic inflammatory diseases of autoimmune origin. In addition, the researchers found a set of genetic variants that is shared among the different chronic inflammatory diseases, which may shed light on a common pathogenic mechanism that causes these diseases.

International MHC and Autoimmunity Genetics Network (IMAGEN). Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. Proc Natl Acad Sci USA 106: 18680-18685, 2009.

BACTERIA AND VIRUSES IN THE HUMAN INTESTINE

Bacterial "Census" Reveals that Healthy People Host Distinct Communities of Microbes: Researchers have developed a catalogue of the diversity and variation in bacterial species that reside on or within the body of healthy people. The human body is host to an enormous ecosystem of microorganisms. This microbial community—or microbiota—contains nearly 100 trillion organisms, with the number of bacterial cells on or in the human body outnumbering human cells by almost 10 to 1. The resident bacterial communities provide important functions that aid in metabolism, help prevent infections, and train the immune system. Since these traits are critical for normal health and may, if altered, contribute to disease, it is important to understand the diversity and variation of the human microbiota at different body sites, among individuals, and over time. Although previous studies revealed the diversity of the bacterial communities residing at distinct body sites, an integrated view of the human microbiota across the entire body is needed to fully define the genetic diversity that contributes to normal health.

Using state-of-the-art DNA sequencing methods, researchers have taken a census of the bacterial communities across several body sites of healthy individuals. The researchers collected samples of the bacterial communities from different body sites—including the gut, mouth, ears, nose, hair, and various skin surfaces—of healthy volunteers on several occasions over a 3-month period. After isolating the bacterial DNA from these samples, the scientists analyzed the DNA sequences of a particular gene known to vary among different bacterial species to determine the diversity of bacterial species present at different sites for each individual. They found that the composition of the bacterial communities was determined mostly by their location on or in the body, with the different body sites having distinct community members. These communities were dominated by four groups of related bacteria, with no one particular group found on all of the body sites of any individual on any given day in this study. In addition, the bacterial community composition at some body sites, such as in the gut, varied considerably between different people. However, each individual's "personalized microbiota" appeared to be relatively stable over time. By defining the composition and variation of the microbiota in healthy individuals, researchers now have a baseline for detecting changes in the microbiota that may be associated with human diseases.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, and Knight R. Bacterial community variation in human body habitats across space and time. Science 326: 1694-1697, 2009.

Exploring the Viruses that Reside in the Human Intestine: Scientists have identified and characterized viruses that live in the human intestines. The Human Microbiome Project (HMP) was initiated by NIH to generate a comprehensive characterization of the microbes that inhabit the human body, as well as their genetic material or genomes—collectively referred to as the microbiome—and to analyze their roles in health and disease. The HMP and other studies of microbes that reside within people have yielded significant information about the content of the human microbiota. These studies have focused primarily on bacteria in the microbiome. Now, scientists have identified the intestinal viral populations in order to explore the role of viruses in relation to the broader microbial community and its human hosts. Most of the viruses identified and characterized in this new study were "bacteriophages," a type of virus that infects bacteria.

The researchers began by isolating viruses from stool samples collected from identical twin sisters and their mothers at three time points over 1 year. A molecular biology technique was used to amplify the small amounts of viruses in the samples. The DNA from the viral populations of each individual's intestine—the "virome"—was then sequenced. The researchers also isolated and sequenced the DNA from bacteria present in the stool samples. By comparing sequences of the viruses and bacteria in the samples with database sequences of known microbes, they were able to identify individual microbial species. In contrast to past research showing that bacterial communities in the intestines of twin pairs and their families were more similar than those of unrelated individuals, analysis of the viral species diversity revealed that the array of viral types was unique to the individual. The diversity of intestinal viruses from each individual was found to be very low and stable throughout 1 year. The most abundant types of bacteria present were those that likely would be infected by the specific forms of viruses identified. The researchers also noted the dominance of types of viruses that infect bacteria, but do not destroy them. Interestingly, this relatively peaceful co-existence differs from the "predator-prey" dynamic of viral-bacterial interactions observed in sludge and other microbial ecosystems that have been studied.

This study attests to the potential benefits of studying intestinal viruses as an important part of research on the human microbiome. Understanding the interactions between intestinal viruses and their bacterial hosts may shed light on the relationships of these microbes with their human hosts. Also, with increased knowledge, the viral component of the intestinal microbial population may serve as a biomarker of microbial response to treatment interventions and progression of disease.

Reyes A, Haynes M, Hanson N, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466: 334-338, 2010.

Immune Cell Surface Protein Links Gut Bacteria to Diet and Protection Against Inflammation: Microbes residing throughout the human body are now being appreciated for their contributions both

to human health and disease. Scientists have shown that the community of microbes living in the human gut has "co-evolved" with its host to the point that a well-balanced gut microbial community is essential for healthy functioning of the digestive system, as well as the immune system, to prevent conditions such as IBD and other inflammatory or autoimmune conditions. For example, gut microbes perform many functions that humans are incapable of, such as harvesting certain nutrients from the foods we consume. Some bacterial species break down dietary fiber into short-chain fatty acids in the large bowel. These fatty acids have been shown to have a beneficial, anti-inflammatory effect on conditions such as IBD, and are known to act directly on cells in the gut or elsewhere by latching onto components of the cell's surface known as "receptors," including one called GPR43 in humans, or Gpr43 in mice.

In this study, researchers aimed to identify the mechanism by which this type of fatty acids, produced as a result of bacterial fermentation of dietary fiber, have a protective effect against IBD and other inflammatory conditions, such as arthritis and asthma. They showed that the presence of intestinal bacteria reduces disease severity using a mouse model that mimics a form of IBD known as ulcerative colitis. For this experiment, they compared mice raised conventionally with those raised in a bacteria-free environment, before and after their guts were repopulated with bacteria. Based on prior knowledge of microbial effects on IBD via production of short-chain fatty acids, which act through receptors like GPR43, the scientists utilized microarray screening technology to identify immune cells that produce high amounts of GPR43/Gpr43 in humans and mice, respectively. In mice genetically engineered to lack Gpr43 and treated to model ulcerative colitis, immune cells did not respond normally to short-chain fatty acids, and these fatty acids did not reduce intestinal inflammation as in wild-type mice. The results of this experiment indicate that short-chain fatty acids act through Gpr43 on the surface of immune cells to exert their protective effect against intestinal inflammation. Similar results were seen in mouse models of arthritis and allergic airway inflammation.

This study identifies how interactions between by-products of bacterial metabolism and a receptor on the surface of immune cells act to protect against IBD and other inflammatory conditions, such as rheumatoid arthritis and allergic inflammation of the airways. These interactions could provide a target for manipulating immune responses in these conditions through such means as diet and prebiotic/probiotic supplementation.

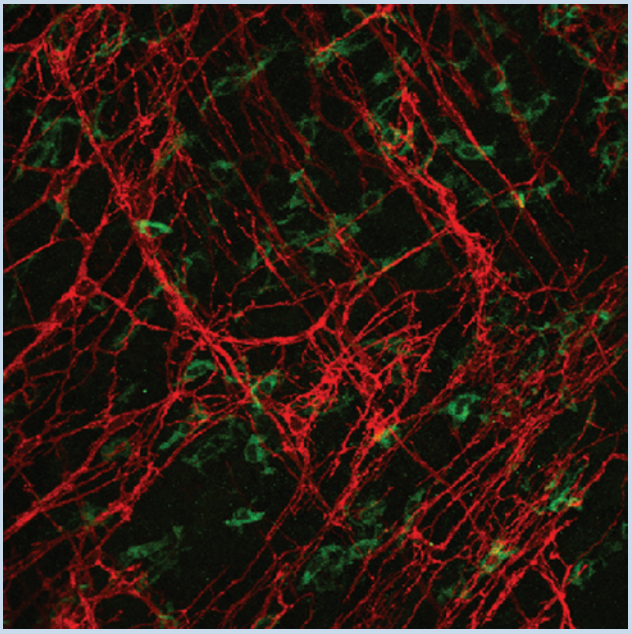
Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461:1282-1286, 2009.

Bench to Bedside Journey for Gastroparesis Treatment

Research supported by NIDDK is translating successes made in the laboratory into a potential new therapy for people with gastroparesis. Gastroparesis is a disorder characterized by delayed emptying of stomach ("gastric") contents into the intestines, due to abnormal function of the muscles of the stomach and intestines. It is referred to as a "motility" disorder, because the muscles do not properly move contents along in the gastrointestinal tract. The muscle dysfunction results from damage to nerves and tissues, which is most commonly caused by diabetes. Retention of food in the stomach leads to such unwelcome symptoms as recurrent nausea, vomiting, bloating, weight loss, and pain in the abdomen.

Through basic research supported by NIDDK since 2004, a group of scientists at the Mayo Clinic in Minnesota have studied the mechanisms underlying gastroparesis. A major focus of their research was on interstitial cells of Cajal (ICCs) that are found throughout the gastrointestinal tract and play an important role in controlling motility. These cells function as intestinal "pacemakers," regulating contraction of muscles in the gastrointestinal tract, similar to a pacemaker for the heart. Gastroparesis is associated with a loss of ICC function. The researchers discovered that they could reverse this disorder in a mouse model by administering an agent that protected the ICCs from oxidative stress associated with diabetic gastroparesis.[1] The agent they used, hemin, is a biological product of red blood cells; it boosts the production of an enzyme called heme oxygenase-1 (HO-1), which helps reduce oxidative stress. Hemin has been used in the past to treat an inherited condition called acute intermittent porphyria.

The investigators subsequently conducted a study in humans demonstrating activation of the HO-1 enzyme in response to a pharmacologically relevant and well-tolerated dose of hemin.[2] Now, they are conducting the first randomized controlled clinical trial to test whether hemin therapy can improve gastric emptying and other symptoms in patients with gastroparesis. The researchers hope that this clinical trial, based on knowledge gained from years of careful labwork, may soon yield an effective medication for gastroparesis. A news story featuring the trial and one of its participants was broadcast in August 2010 on an ABC News affiliate in Minnesota.



This image shows the network of cells, called ICCs (interstitial cells of Cajal), from the stomach of a mouse. The ICCs (shown in red) reside in muscle layers of the stomach and exist in close relationship with immune cells called macrophages that produce the enzyme HO-1 (in green). HO-1 protects the nearby ICCs from damage. Reduced levels of HO-1 result in loss of ICCs and of key neuronal factors, leading to the development of gastroparesis. Scientists are testing whether therapy with the biological agent hemin can help people with gastroparesis through increased production of HO-1, thereby allowing repair of the ICC network, re-expression of key neuronal factors, and normalization of gastric emptying. Image provided by Dr. Gianrico Farrugia.

In addition to supporting the ongoing work of this group of investigators, NIDDK sponsors other research on gastroparesis through its Gastroparesis Clinical Research Consortium. The Consortium performs clinical research to develop effective treatments for this disorder. The Consortium's studies include multi-center clinical trials testing existing pharmaceuticals, such as the antidepressant nortriptyline, as potential treatments to improve gastroparesis symptoms. Additional information on the Consortium is available at: *www.jhucct.com/gpcrc/default.asp*

[1] *Choi KM, et al. Gastroenterology 135: 2055-2064, 2008.*

[2] *Bharucha AE, et al. Clin Pharm Ther 87: 187-190, 2010.*

BIOENGINEERING APPROACHES TO TREATING FECAL INCONTINENCE

A Potential Step Toward Treating Fecal Incontinence—Functioning Bioengineered Anal Sphincter Implants in Mice: In research that may have implications for future development of treatment for fecal incontinence, scientists have successfully sustained physiologically functional, implanted bioengineered internal anal sphincters (IAS) in mice. The IAS is a ring-like muscle located at the end of the rectum. An incompetent IAS muscle is a primary cause for the uncontrolled release of stool that occurs in people with fecal incontinence, a condition that places devastating emotional, social, physical, and economic burdens on people who are affected by it.

In a recent study, scientists used smooth muscle cells obtained from mouse IAS to bioengineer three-dimensional IAS rings. The cells were grown on special plates containing a mold around which the cells could create the three-dimensional ring structure. Once an IAS was formed, it was implanted into a small pocket made surgically under the skin of a recipient mouse. The key challenge for the scientists in this study was to promote the development of a vascular network that would supply blood to the implant, ensuring its survival over an extended period of time. In an effort to stimulate the growth of new blood vessels, the researchers placed a tiny pump with a catheter alongside the nascent IAS in the surgical pocket. The pump was able to provide a constant flow of a growth factor called FGF-2 to the new implant and surrounding tissue. Examination of the implants and surrounding tissue 25 days after surgery revealed that the scientists' approach was successful. The IAS implants that continually received growth factor thrived. New blood vessels emerged in the surrounding tissue, the bundles of muscle cells forming the IAS structure appeared healthy, and there was no evidence of rejection of the implants, which had been constructed using donor mice cells.

In a subsequent study, the scientists compared the physiological functions of bioengineered IAS before and after they were implanted in mice for 4 weeks. IAS were first tested for spontaneous basal tension (the state which prevents uncontrolled release of stool) by attaching one end of the IAS to a stainless steel pin fixed to the center of a culture plate containing a physiological solution and attaching the other end, without stretch, to a movable tension measuring arm. To test functionality, the IAS was bathed in solutions to stimulate or relax the IAS muscle, and the force exerted on the tension arm was measured. By comparing the tensions for pre- and post-implantation IAS, the researchers demonstrated that the implanted IAS had retained physiological integrity. The bioengineered muscles were able to contract and relax in a manner that mimicked the natural *in vivo* function of the IAS.

This study's successful implantation in mice of bioengineered IAS that retained physiological functionality presents an opportunity for new studies that one day may be translated into bioengineered IAS for people suffering from incontinence. This would be an enormous benefit for these people, greatly improving their daily lives and alleviating the social and financial burdens associated with this disorder.

Hashish M, Raghavan S, Somara S, et al. Surgical implantation of a bioengineered internal anal sphincter. J Pediatr Surg 45: 52-58, 2010.

Raghavan S, Miyasaka EA, Hashish M, et al. Successful implantation of physiologically functional bioengineered mouse internal anal sphincter. Am J Physiol Gastrointest Liver Physiol 299: G430-G439, 2010.

CELIAC DISEASE RESEARCH

New Genetic Variants Associated with Celiac Disease Are Identified: Scientists have uncovered new genetic variants that are associated with the risk of celiac disease and have linked these variants to four pathways of the immune system. Celiac disease is a complex genetic disease that can cause damage to the intestine, resulting in poor absorption of nutrients, painful digestive and other symptoms, and other serious complications. These symptoms occur when people with the disease eat grains containing gluten—such as from wheat, rye, and barley—which provokes an abnormal immune response that attacks their intestine. For children, celiac disease can have devastating consequences, such as impaired growth and development, while adults may experience anemia, bone loss, and other complications.

In an earlier study, scientists conducted a genome-wide association (GWA) study to identify two gene variants that are required for celiac disease, and 12 chromosome regions that are associated with a risk for the disease. Although these findings were impressive, it was determined that all of the known variants did not account entirely for the genetic risk of celiac disease. In the new study, scientists set out to identify variants that may have smaller, yet critical, effects on disease risk. This was accomplished with a larger GWA study that included DNA samples from a larger number of patients with celiac disease and healthy volunteers. The samples were analyzed using a denser concentration of probes to identify differences in the DNA sequences of the patients compared with those of the volunteers. This approach was successful in uncovering 13 new chromosome regions that are associated with celiac disease, and 13 additional chromosome regions with suggestive associations with celiac disease. Many of these regions were found to contain genes with functions related to the immune system. In addition, uncovering the genetic variants led the scientists to identify four specific immunological pathways that are relevant to the pathogenesis of celiac disease. The scientists also found that more than half of the variants associated with celiac disease correlate with the extent to which nearby genes are turned on or turned off (expressed), indicating that the variants may increase the risk of celiac disease by influencing the expression of other genes. These new findings have advanced knowledge of celiac disease and may also have important implications for other autoimmune diseases, such as type 1 diabetes.

Dubois PC, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. Nat Genet 42: 295-302, 2010.

Mixed Effects of Undiagnosed Celiac Disease in Older Adults: Scientists studying the consequences of undiagnosed celiac disease in a population of American men and women 50 years of age and older found that undiagnosed celiac disease did not increase the risk of death over the 10-year period of the study, although other health consequences were observed. Celiac disease is an autoimmune disease caused by intolerance of the gluten proteins found in many grains. Although there is no cure for celiac disease, it can be treated effectively with a gluten-free diet. Previous research findings from different studies have differed with respect to whether undiagnosed celiac disease increases rates of premature death. Now scientists have surveyed a group of older men and women in the general population over a 10-year time period to determine how people who are 50 years of age or older may be affected by undiagnosed celiac disease.

For this study, scientists screened frozen blood samples from almost 17,000 people living in Olmsted County, Minnesota, using assays for particular antibodies that are characteristic of celiac disease. People who had not been diagnosed with clinical celiac disease but whose blood samples tested positive for the disease with two different antibody assays were classified as having undiagnosed celiac disease. Blood samples that tested negative were used for the study's control group. Analysis of the data from this screening determined that approximately 0.8 percent of the people whose blood samples were screened had undiagnosed celiac disease. The medical records of the undiagnosed celiac group and the control group were then reviewed for more than 100 potential medical conditions, or cases of death, over the 10-year period after the blood samples had been collected. The records showed that, among the people whose blood samples had tested positively, approximately 15 percent subsequently received a clinical diagnosis of celiac disease. In contrast, no individuals in the control group were diagnosed with celiac disease. This study did not find an increase in mortality among people with undiagnosed celiac disease, although other health risks and potential benefits were observed in this group. The undiagnosed celiac group had increased risk of osteoporosis and hypothyroidism, but they also had lower BMIs (body mass index) and cholesterol levels.

The aim of this study was to determine the effects of undiagnosed celiac disease on older men and women. However, the mixed study results do not clarify whether awareness of undiagnosed celiac disease in cases where there are no clinical symptoms provides a net benefit to the individual. It thus remains unclear whether screening of the general public for celiac disease is warranted.

Godfrey JD, Brantner TL, Brinjikji W, et al. Morbidity and mortality among older individuals with undiagnosed celiac disease. Gastroenterology 139:763-769, 2010.

PREDICTORS, GENETICS, AND TREATMENT OF NONALCOHOLIC FATTY LIVER DISEASE

Identifying Noninvasive Predictors of Nonalcoholic Fatty Liver Disease: Using a database of demographic, clinical, and laboratory information on patients with nonalcoholic fatty liver disease (NAFLD), scientists have evaluated the utility of these noninvasive variables to diagnose and predict severity of nonalcoholic steatohepatitis (NASH)—a more serious form of NAFLD that is accompanied by liver injury, inflammation, and fibrosis. Currently, a liver biopsy, where a physician removes a sample of liver tissue to examine under a microscope, is the only definitive way to diagnose NASH and to identify disease severity. However, a liver biopsy is an invasive procedure that has challenges with patient acceptance, costs, and sampling variability, as well as risks associated with it being an invasive test. It would, therefore, be beneficial to develop noninvasive methods for diagnosing NASH using routinely obtained clinical and laboratory data.

The NIDDK-supported NASH Clinical Research Network has collected clinical and laboratory information on 1,266 adults with diagnosed or suspected NAFLD who have participated in the Network's clinical studies. As part of these studies, most of the participants also received liver biopsies. Of the study participants who received a liver biopsy, 57 percent were diagnosed with having "definite" NASH. By analyzing the demographic, clinical, and laboratory data for these individuals, the scientists found that patients with NASH were more likely to be women, have diabetes, and show signs of metabolic syndrome. There was not a significant difference in age, measures of obesity, and ethnicity for those with biopsy-diagnosed NASH compared to those without. In addition, measures of liver enzyme levels, which are common laboratory tests for assessing liver function and potential liver damage, did not appear to be useful screening tools for diagnosing NASH in patients with NAFLD. However, liver enzyme levels were reliable measures for predicting the most advanced stages of liver injury resulting from NASH. To develop a tool for diagnosing the presence and severity of NASH, the researchers developed a predictive model based on the demographic, clinical, and laboratory information available. As more information was included in the model, the researchers were better able to predict advanced stages of liver injury in adults with NAFLD.

Researchers in the NASH Clinical Research Network will continue to follow these patients to better understand the causes and natural history of NAFLD. By identifying additional clinical or demographic "markers" associated with disease, researchers may be able to develop more robust, noninvasive measures for predicting the presence and severity of NASH.

Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 52: 913-924, 2010.

Metabolic Syndrome Is a Potential Predictor of Liver Damage in Children: A new study indicates that criteria for metabolic syndrome may identify children who are at greatest risk for severe liver damage caused by NAFLD. NAFLD is caused by the accumulation of fat in the liver of people who drink very little or no alcohol. It is the most common cause of pediatric chronic liver disease in North America. In adults, NAFLD has been associated with metabolic syndrome, a constellation of symptoms that places people at risk for cardiovascular disease and type 2 diabetes. Recent studies suggest that this association may also place children at risk. In response to these findings, scientists conducted a study to analyze the metabolic syndrome-NAFLD relationship in children and adolescents.

Overweight and obesity are the primary risk factors for NAFLD and are part of metabolic syndrome. Other components of metabolic syndrome include high blood pressure, low HDL (good) cholesterol, high triglycerides, and insulin resistance (an indicator of type 2 diabetes or diabetes risk). The children and adolescents who participated in the current study were all enrolled in the NASH Clinical Research Network, a multi-site clinical network established by NIDDK to assess the causes, natural history, and therapy of NAFLD. The scientists conducting this study used liver biopsies to assess liver damage and clinical tests to measure the components of metabolic syndrome. Children in the study were diagnosed with metabolic syndrome if they had three of the five criteria mentioned above. Everyone who participated in the study had NAFLD, and 25 percent were found to have metabolic syndrome as well. Deeper analyses of the comparisons of the frequency and severity

of symptoms of NAFLD and metabolic syndrome revealed important correlations between them. Scientists used several criteria for diagnosing the severity of liver disease, including the amount and pattern of liver fat that was present, ballooning or enlargement of liver cells, and the degree of liver tissue scarring. Analyses of these conditions demonstrated that all of these criteria were significantly associated with metabolic syndrome. Central obesity (large waist circumference) and insulin resistance, however, were the features that were most consistently associated with the severity of liver damage caused by NAFLD.

This study uncovered important relationships between metabolic syndrome and liver damage that results from pediatric NAFLD. If future clinical studies confirm this correlation, these relatively noninvasive methods—compared to liver biopsy—may be used to evaluate the risk and progression of liver disease in children and adolescents with NAFLD.

Patton HM, Yates K, Unalp-Arida A, et.al. Association between metabolic syndrome and liver histology among children with nonalcoholic fatty liver disease. Am J Gastroenterol 105:2093-2102, 2010.

Genetic Variants Associated with Nonalcoholic Fatty Liver Disease: Two large studies using data from the NASH Clinical Research Network and other sources have found associations between genetic variants and NAFLD diagnosed by liver biopsy. In recent years, NIDDK-supported investigators have conducted genome-wide association studies to identify genetic factors that could predispose some individuals to develop NAFLD. For example, researchers uncovered a variant in a gene called patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), which codes for a type of enzyme involved in lipid metabolism. A variant of *PNPLA3* is known to be associated with elevated liver enzymes and with excess fat in the liver, which serve as noninvasive markers of NAFLD. However, the gold standard test for diagnosing NAFLD remains the liver biopsy, in which key structural changes characteristic of the disease can be observed under the microscope. Two research groups recently conducted studies that tested whether these genetic variants were associated with NAFLD, when it was diagnosed using the more definitive test of a liver biopsy.

In one study, scientists used genomic data from adults and children participating in the NASH Clinical Research Network, as well as patients with NASH who participated in clinical studies at the NIH Clinical Center. The majority of the study participants were Caucasian. The researchers determined whether these individuals carried any of the six genetic variants previously associated with elevated liver enzymes and excess liver fat. Of these variants, the *PNPLA3* variant was most strongly associated with several features of NAFLD observed in the liver biopsy. In children with NAFLD, the *PNPLA3* variant was associated with earlier disease development. The study also associated the *PNPLA3* variant and other genetic variants with more severe forms of NAFLD in adults.

In a separate study, another group of researchers used genomic data and samples collected by the Network and other consortia to test whether genetic variants were associated with biopsy-confirmed NAFLD, as well as features of metabolic syndrome. They chose to focus on seven genetic variants that had been associated with NAFLD in previous studies using liver imaging of excess liver fat and liver function tests. For data on individuals with NASH, they used samples from the NASH Clinical Research Network, which they compared to samples from ancestry-matched controls studied through the Myocardial Infarction Genetics Consortium, which is supported by the National Heart, Lung, and Blood Institute. The study also focused on individuals of European ancestry who participated in these consortia, in order to reduce genetic variability in the study population. By analyzing genomic data from these sources and associating it with cases of NAFLD confirmed by microscopic evaluation of liver biopsies, the researchers found that out of the seven genetic variants analyzed, only the *PNPLA3* variant was strongly associated with the disease and its severity. Additionally, using data from consortia conducting genome-wide association studies related to elevated blood lipids, type 2 diabetes, and obesity, the group showed that this gene variant was specifically associated with NAFLD, but not with aspects of metabolic syndrome.

These studies demonstrate a strong association between gene variants such as *PNPLA3* and biopsy-confirmed NAFLD. Genetic analyses such as these shed light on the multiple metabolic pathways involved in this form of liver disease, which may lead to the development of

therapies targeting these pathways. Additional studies of NAFLD genetics are needed that involve other racial/ethnic groups to identify genetic factors that could contribute to differences observed in NAFLD prevalence.

*Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, and the NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) with histological severity of nonalcoholic fatty liver disease. Hepatology 52: 894-903, 2010.*

Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 *variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology 52: 904-912, 2010.*

Identification of a Major Genetic Risk Factor for Nonalcoholic Fatty Liver Disease: Researchers have identified genetic variants that impart a high risk of NAFLD, a common condition that is associated with risk for more serious liver disease, as well as type 2 diabetes, cardiovascular disease, and liver cancer. NAFLD—essentially the abnormal accumulation of fatty deposits in the liver in the absence of chronically high alcohol intake—is most prevalent in obese individuals, and is therefore linked to excessive consumption of calories. However, many people with obesity do not have NAFLD, while some people of normal weight do. Most people with NAFLD experience no symptoms early in the course of the disease. The fat deposits observed in NAFLD are comprised primarily of a group of molecules called triglycerides. In the blood, triglycerides are typically associated with a class of proteins called apolipoprotein C. Previous research had shown that two variants in the *APOC3* gene, which encodes one of these proteins, were associated with elevated blood levels of triglycerides.

To test whether these *APOC3* variants affect the likelihood of NAFLD, researchers studied a group of 95 Asian Indian men who were sedentary, but neither obese nor alcoholic, and who had not been previously diagnosed with disease. The researchers found that 38 percent of the men who had at least one of the two high triglyceride *APOC3* variants also had NAFLD, while none of the men who lacked both variants had the disease. This was a strong indication that *APOC3* has a significant impact on risk of NAFLD in Asian Indian men. To further assess the impact of *APOC3* on risk for NAFLD, the researchers then examined a group of 163 apparently healthy men from other ethnic groups. They found that 9 percent of those with the high risk variants actually had the disease, but again, found no NAFLD in the men without them. The researchers also observed insulin resistance—which is known frequently to lead to type 2 diabetes—in the men who had NAFLD, but not in the men without the disease, whether or not they had the high risk *APOC3* variants. Encouragingly, when the researchers provided a 3- to 6-month dietary intervention to seven of the Asian Indian men who had both NAFLD and insulin resistance, they found that liver fat content fell and insulin resistance abated—suggesting that a healthful lifestyle can help prevent NAFLD and insulin resistance even in people with high risk forms of *APOC3*. These results shed light on the genetic basis for NAFLD, while providing hope that this common liver disease may be preventable even in those at high genetic risk.

Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 362: 1082-1089, 2010.

Largest Trial in Adults with Nonalcoholic Steatohepatitis Shows Benefits of Treatments: NASH is a serious and increasingly common form of NAFLD in the U.S. Its features include excess fat and inflammation in the liver similar to alcoholic liver disease, but they occur in people who drink little to no alcohol. Though its precise causes are unknown, it is typically associated with obesity, type 2 diabetes, hypertension, elevated lipids in the blood, or other features of metabolic syndrome, which affects a growing number of adults and children in the U.S. However, NASH can also affect people who are of normal weight and do not have diabetes or other signs of metabolic syndrome. The disease often goes undetected for years, until an abnormality in liver function unrelated to other common causes of liver disease is noticed incidentally through a measure such as elevated liver enzymes in the blood. Long-term NASH may develop into severe cirrhosis, liver cancer, and/or liver failure requiring a transplant. Currently, there are no specific, Food and Drug Administration-approved treatments available for NASH.

To test potential treatments for NASH in adults, NIDDK's NASH Clinical Research Network conducted a clinical trial called the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis trial, or "PIVENS." This multi-center clinical trial tested whether treatment with pioglitazone, an insulin-sensitizing drug used for diabetes, or the antioxidant vitamin E could improve NASH in non-diabetic adults, in comparison to placebo. Non-diabetic adults were studied in order to test the insulin-sensitizing drug in a population that had not already been treated with this type of drug. Participants were treated with a once-daily dose of vitamin E, pioglitazone, or placebo for 2 years, followed by 6 months of evaluation after treatment. Liver biopsies and other clinical samples were taken to assess whether features of NASH, such as fibrosis, inflammation, and fat in the liver, improved during the course of treatment. Although only vitamin E significantly improved an overall measure of NASH, both vitamin E and pioglitazone improved some of the features of this disease, including a reduction in liver enzyme levels, as well as fat and inflammation in the liver. However, many participants taking pioglitazone also experienced the unhelpful side effect of weight gain.

The PIVENS trial is the largest randomized controlled clinical trial to date in patients with NASH. The results from this trial represent an important milestone in the search for effective treatments for this common form of liver disease. While these results are promising, especially for vitamin E, patients should consult a physician before using such high-dose vitamin E or pioglitazone long term for the treatment of NASH. Future research will be needed to determine whether the benefits of vitamin E and pioglitazone extend to adults with both diabetes and NASH, or will continue with minimal risks during long-term treatment. Some trial participants have chosen to continue participating in the Network's prospective, longitudinal follow-up study, so that they can continue to contribute to our knowledge of this disease and its long-term management.

Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. New Engl J Med 362: 1675-1685, 2010.

For additional information on the PIVENS trial and other NASH research efforts, please see the Patient Profile and Story of Discovery in this chapter.

ADVANCES ON RARE FORMS OF GENETIC LIVER DISEASES

A Promising Treatment for Alpha-1 Antitrypsin Deficiency: Researchers have used modified cell lines and mouse models mimicking the conditions of alpha-1 antitrypsin deficiency to show dramatic benefits of a drug already approved for other indications, which works by activating a cellular process called "autophagy." Alpha-1 antitrypsin deficiency is the most common form of genetic liver disease in children, and a cause of cirrhosis and liver cancer in adults as well. This disease is the result of a mutation in the alpha-1 antitrypsin (alpha-1 AT) protein, which is produced in the liver and released into the blood to exert a protective effect on the lungs. This mutation causes the alpha-1 AT protein to have an aberrant, misfolded shape and to be retained inside the liver cells, where it accumulates and damages the liver. Basic research has shown that liver cells have some limited defenses against the mutated protein in the form of pathways that degrade unnecessary or abnormal proteins; one of these is the autophagy pathway. Based on this knowledge of mechanisms by which the mutated alpha-1 AT protein is degraded by processes such as autophagy, researchers set out to test whether a drug that boosts autophagy could effectively treat the liver disease associated with alpha-1 antitrypsin deficiency.

Starting from a list of drugs that were recently shown to enhance autophagy of proteins that, like mutated alpha-1 AT, accumulate in cells, researchers selected the drug carbamazepine based on its extensive safety profile in humans. Carbamazepine is currently prescribed as an anticonvulsant and mood stabilizer. The researchers first tested carbamazepine in a human cell line that was genetically altered in the laboratory to produce a mutated form of the alpha-1 AT protein. The drug markedly reduced the amount of accumulated protein in the cells by enhancing its degradation. Further testing showed that the drug acted by boosting autophagy beyond the cell's usual response to accumulated protein. Additional testing in mouse cell lines that were modified to inactivate autophagy or proteasomal degradation pathways indicated that both pathways are used to some extent by carbamazepine to enhance disposal of the mutant alpha-1 AT protein. The researchers then turned to a mouse model that mimics liver disease associated with alpha-1 antitrypsin

deficiency. Mice had been genetically altered to produce the human mutated alpha-1 AT protein, as well as produce a green fluorescent protein in cells where autophagy was occurring to allow easy detection of signs of this cellular process under the microscope. Two weeks of treatment with carbamazepine in this mouse model decreased levels of the mutated protein in the liver, where signs of ramped-up autophagy were found. In these mice, the treatment also reduced liver fibrosis, a primary feature of liver disease associated with alpha-1 antitrypsin deficiency.

This study demonstrates the power of combining basic research on cellular processes underlying disease with knowledge of existing therapeutics that target these processes in order to identify promising treatments that may work for multiple diseases. These pre-clinical studies in mice show the potential of the autophagy-enhancing drug carbamazepine as a treatment for liver disease associated with the genetic liver disease alpha-1 antitrypsin deficiency. However, future clinical studies will be needed to test the benefits of this treatment in pediatric and adult patients with the disease.

Hidvegi T, Ewing M, Hale P, et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science 329: 229-232, 2010.

Viral-Mediated Gene Repair Corrects Hereditary Tyrosinemia Mutation in Mouse Liver: Scientists have successfully repaired a mutation in the gene linked to the metabolic disease hereditary tyrosinemia type 1 (HT1) in mice using a specially engineered virus to transport the normal gene sequences, thereby preventing and ameliorating the associated liver damage. HT1 is a rare and fatal metabolic disease caused by genetic mutation leading to deficient activity of the enzyme fumarylacetoacetase. This enzyme catalyzes the last step in the cellular pathway that breaks down the amino acid tyrosine, which is a component of proteins and can be obtained from food. In humans with insufficient activity of this enzyme, metabolic compounds in the pathway build up in liver and kidney cells to toxic levels, causing cell death. Liver damage in patients with HT1 can range from acute liver failure during the first months of life to a progressive chronic liver disease after the first years of life. Therapeutic options for this condition are limited to dietary restriction of tyrosine, liver transplantation, or treatment with an agent called NTBC, which interrupts a different part of the tyrosine metabolic pathway in a way that prevents the toxic compounds from accumulating. However, none of these approaches are optimal or are capable of addressing the underlying enzyme deficiency before severe damage to the liver and other organs occurs. Developing a strategy to repair the underlying genetic defect in patients with HT1 has the potential to directly treat or even prevent this disease.

To test their treatment strategy, the research team used a mouse model with a mutation in the gene coding for the fumarylacetoacetase enzyme, which develops a disease similar to human HT1, but in an accelerated manner. They also designed a tool for gene repair consisting of an adeno-associated virus fused to a segment of the normal gene that encodes the enzyme. Adeno-associated viruses had previously been studied in animal models and humans as potential vehicles for delivering gene therapy. The normal gene sequences carried by the virus can be used by a cell to replace the mutation in its native, mutant gene, thus enabling production of the normal enzyme and treatment of HT1. Neonatal and adult mice with the mutated enzyme were given NTBC to protect against fatal liver injury and injected either with their virus-based gene repair kit or with saline. The scientists then stopped administering NTBC and observed signs that the gene for the defective enzyme had been successfully repaired in mutant mice given the virus carrying the normal gene. For example, the liver cells of the mice began to produce the corrected enzyme and even show a growth advantage as they repopulated the liver with healthy cells. Liver function tests demonstrated that the underlying liver disease was almost completely corrected by gene repair in the mutant mice.

This study provides a proof of principle in an animal model for the use of gene repair to correct an inherited metabolic liver disease. With additional research, targeted gene repair using the adeno-associated virus may prove to be an effective treatment for HT1 and other similar diseases resulting from mutation of a single gene.

Paulk NK, Wursthorn K, Wang Z, Finegold MJ, Kay MA, and Grompe M. Adeno-associated virus gene repair corrects a mouse model of hereditary tyrosinemia in vivo. *Hepatology 51: 1200-1208, 2010.*

TREATMENT FOR ACUTE LIVER FAILURE

Successful Treatment for Early-Stage Acute Liver Failure Not Caused by Acetaminophen: Results of a recent clinical trial testing a new treatment for patients in the early stages of acute liver failure (ALF) due to causes other than acetaminophen overdose showed improved outcomes with the treatment. ALF is a rare but devastating condition for which the only therapy currently available is liver transplantation. The majority of ALF cases in the U.S. are caused by toxicity from an overdose of the over-the-counter pain reliever acetaminophen. Fortunately, cases of acetaminophen-related ALF can be successfully treated if caught in the early stages, with an agent called N-acetylcysteine (NAC), which neutralizes a toxic product of acetaminophen metabolism. Researchers speculated that this antidote might have beneficial properties that could prove useful in treating cases of ALF resulting from other causes, including other forms of drug-related injury, autoimmune hepatitis, and hepatitis B.

The NIDDK's Acute Liver Failure Study Group conducted a clinical trial across 24 U.S. sites to test whether NAC treatment could improve survival and reduce the need for liver transplantation in patients with ALF from causes other than acetaminophen toxicity. After patients were given an intravenous infusion of NAC or placebo for 72 hours, survival and transplantation rates were assessed 3 weeks and 1 year later. Results were compared across groups of patients based on their stage of disease prior to treatment. Although no significant differences in overall survival emerged, patients with less advanced disease who were given NAC showed improved survival without the need for a transplant, compared to those given placebo. NAC was also well-tolerated, with uncommon and minor side effects.

Based on the results of this trial, NAC shows promise as a safe and effective treatment for early-stage, non-acetaminophen-related ALF, a condition for which no other therapeutic option currently exists beyond liver transplantation. Future studies may explore optimal dosing and duration of this treatment, as well as predictors of patient response and the physiologic basis of this response, in order to achieve the greatest benefit from this treatment for ALF resulting from causes other than acetaminophen toxicity.

Lee WM, Hynan LS, Rossaro L, et al. Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. Gastroenterology 137: 856-864, 2009.

COMPLICATIONS OF ANTIVIRAL THERAPY IN CHILDREN WITH HEPATITIS C

Infrequent Eye-Related Complications from Treatment of Chronic Hepatitis C in Children: Researchers have determined that eye-related (ophthalmologic) complications resulting from standard antiviral therapy for chronic hepatitis C are uncommon in children, contrasting with the higher adult rate of these complications.

The standard therapy for chronic hepatitis C—a combination of antiviral drugs known as peginterferon and ribavirin—has been shown in adults to be effective at suppressing viral infection. However, peginterferon has also been associated with adverse effects, including eye-related complications, such as retinopathy. Much less had been known about how children with chronic hepatitis C respond to antiviral therapy in terms of outcomes and complications. The Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C (Peds-C) Study was a prospective, randomized controlled clinical trial to assess outcomes of peginterferon therapy, given with either ribavirin or placebo, in children with chronic hepatitis C. While the main trial was completed in 2008, a long-term follow-up study of children participating in the trial is currently underway.

Recently, scientists in the Peds-C Study Group collected information on eye-related complications in children with chronic hepatitis C who were treated with peginterferon, with or without ribavirin. The children underwent periodic eye exams before and after treatment. The researchers found that the prevalence of eye-related complications, such as retinopathy, is low in these children, particularly in comparison with the higher rates observed in adults treated for chronic hepatitis C. These results provide information that is useful to health care providers caring for children with chronic viral hepatitis C who are treated with antiviral therapy. While eye-related complications appear to be relatively uncommon for

these children, the severity of these complications when they do occur requires that they continue to be monitored for their occurrence.

Narkewicz MR, Rosenthal P, Schwarz KB, et al. Ophthalmologic complications in children with chronic hepatitis C treated with pegylated interferon. J Pediatr Gasteroenterol Nutr 51: 183-186, 2010.

GENETICS OF PRIMARY BILIARY CIRRHOSIS

New Genetic Risk Factors Discovered for Primary Biliary Cirrhosis: Researchers have identified new genetic variants and confirmed previously identified genetic risk factors that are associated with primary biliary cirrhosis (PBC). PBC is a chronic autoimmune disease characterized by inflammation and damage to the bile ducts, which may ultimately lead to liver damage, cirrhosis, and end-stage liver disease. This disease is believed to be triggered by an autoimmune response in which the body's immune system inadvertently attacks and destroys specific cells lining the bile ducts. As is the case with many other autoimmune diseases, PBC has a strong genetic component. Researchers previously identified genetic variants in three specific regions of the genome that are more frequently found in people with PBC than in healthy individuals. These regions contain genes that are involved in mediating inflammatory responses.

In a new study, researchers have confirmed these genetic associations in a new population of individuals with PBC. In addition, by combining their results with those of the original genome-wide association study, the researchers were able to identify genetic risk factors in three additional regions of the genome that are associated with PBC. One region—the *IRF5-TNPO3* locus—is of particular interest because of its integral role in regulating immune responses. After determining the DNA sequence of this region in patients and healthy individuals, the researchers identified two variants that account for nearly all of the association with PBC in this genomic region. These variants have been associated with other autoimmune diseases and are known to affect the activity of a gene involved in mediating immune responses. This study also identified two other gene regions with variants that increase risk for PBC and have been associated with other autoimmune diseases, including type 1 diabetes, Crohn's disease, celiac disease, and rheumatoid arthritis.

By defining the genetic variants associated with disease, researchers may be able to gain insight into the molecular factors that trigger the onset and progression of PBC. These insights could possibly be extended to other diseases as well, since there appears to be considerable genetic overlap between the risk for PBC and other chronic autoimmune diseases.

Hirschfield GM, Liu X, Han Y, et al. Variants at IRF5-TNPO3, *17q12-21, and* MMEL1 *are associated with primary biliary cirrhosis. Nat Genet 42: 655-657, 2010.*

STORY OF DISCOVERY

Rise of a "Hitherto Unnamed" Liver Disease—Nonalcoholic Steatohepatitis

In recent decades marked by an obesity epidemic in the U.S., researchers have witnessed the rise of a form of liver disease that now appears to affect more American adults, as well as a growing number of the nation's children, than any other—nonalcoholic fatty liver disease. This form of liver disease includes a more severe condition known as nonalcoholic steatohepatitis. Researchers are greatly advancing progress in understanding this disease and in developing potential treatment strategies.

In the 1950s, studies of obese veterans first documented a new form of liver disease characterized by excessive fat in the liver that occurred in individuals who did not report heavy alcohol consumption or have any other known causes of liver injury. However, cases such as these were largely dismissed as related to presumed hidden alcohol abuse. A report in 1980 described several obese patients with diabetes who, though they also did not abuse alcohol, had fat accumulation in the liver accompanied by liver inflammation—indicators that, at the time, were associated only with alcoholic hepatitis. By this point, clinical investigators had begun to realize that people with this condition really were not abusing alcohol. The investigators thus coined the term "nonalcoholic steatohepatitis" (or NASH) to describe what they referred to as a "hitherto unnamed" form of liver disease.

Understanding Disease Processes and Development

The NIDDK sponsored early research on this newly recognized form of liver disease to identify the biologic processes involved and chart the course of disease development and progression. In the 1990s, studies of liver biopsies from obese patients with or without diabetes identified some of the key morphologic changes that take place in NASH. They also charted disease progression in these patients, some of whom developed scarring, or fibrosis, which can progress to cirrhosis. Furthermore, these studies pointed to a link between NASH and insulin resistance, a condition that is also associated with type 2 diabetes. Additional studies in the early 2000s confirmed the link between NASH and insulin resistance, as well as other metabolic abnormalities, such as increased fatty acid oxidation and oxidative stress in the liver. NASH is now thought to be part of a spectrum of nonalcoholic fatty liver disease (NAFLD), which includes simple steatosis (excess fat in the liver) that, with time in some cases, can develop inflammation and other cellular changes characteristic of NASH.

Determining Prevalence and Risk Factors

In 2003, NIDDK supported one of the first population studies to estimate the prevalence of NAFLD, including NASH, in the U.S. using data from the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey. In this study, major risk factors associated with these diseases in the U.S. included overweight with central distribution of fat (in the abdomen), as well as elevated insulin levels indicative of insulin resistance. However, researchers were also learning that, while NAFLD is often associated with obesity, there are cases of normal weight individuals with the disease. This research and subsequent studies supported by the Institute have shown that the prevalence of NAFLD/NASH varies widely among different ethnic and gender groups, with Hispanics and some Asian sub-groups, such as Asian Indian men, having a higher prevalence, African Americans having a lower prevalence, and Caucasians in between. In the mid-2000s, research by NIDDK grantees found that NAFLD was also found in a large number of American children and adolescents, with similar racial/ethnic differences observed.

STORY OF DISCOVERY

Finding Treatments

Building on the earlier research identifying risk factors that contribute to NASH/NAFLD, such as insulin resistance and oxidative stress, NIDDK-sponsored investigators conducted some of the first clinical research to identify potential therapies for this disease. In the 2000s, NIDDK intramural and extramural scientists conducted pilot studies of therapies for NASH, including the insulin-sensitizing drugs metformin and pioglitazone, as well as the antioxidant vitamin E, which improved NASH after short-term treatment. Progress in developing animal models to define disease mechanisms and test new treatments for NASH/NAFLD included studies of a mouse model deficient in serotonin, which provided evidence that this chemical may serve as a future target for NASH therapy.

In 2002, NIDDK created the NASH Clinical Research Network to assemble a large, well-characterized study population for clinical research on the causes, natural history, complications, diagnosis, and therapy of NAFLD, particularly NASH, in both children and adults. Collaborators supporting the Network have included the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Institute on Minority Health and Health Disparities, and the pharmaceutical industry, which provided some of the funding and medications used in clinical trials. Building on the earlier results from pilot studies, in the late 2000s, the Network conducted the largest randomized controlled clinical trial to date of adult NASH therapy, called the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis trial, or "PIVENS." PIVENS showed promising improvements in aspects of adult NASH in response to 2-year therapy with the natural form of the antioxidant vitamin E and the insulin-sensitizing drug pioglitazone. (For additional information on the PIVENS trial, please see a related research advance and Patient Profile in this chapter.) A similar clinical trial in children conducted by the Network, called the Treatment of Nonalcoholic Fatty Liver Disease in Children trial, or "TONIC," tested whether 2-year treatment with the natural form of vitamin E, the insulin-sensitizing drug metformin, or placebo improved pediatric NAFLD. In an early announcement of trial results presented at the 2010 meeting of the American Association for the Study of Liver Diseases, although neither vitamin E nor metformin treatment normalized liver enzymes in children with NAFLD compared to placebo, vitamin E treatment did improve some important features of NAFLD and may reverse NASH in children.

The NASH Clinical Research Network is planning additional studies in adults and children, including a trial to evaluate whether treatment with a drug called obeticholic acid, which is derived from a human bile acid, is a safe and effective treatment for adult NASH.

The Network is also conducting an ongoing, prospective follow-up study of participants in the PIVENS and TONIC trials to collect additional information on NASH/NAFLD in both adults and children. Data and samples collected by the Network are available for ancillary studies by the wider research community.

Identifying Genetic Factors

In recent years, NIDDK-sponsored investigators have identified genetic factors that could predispose some individuals to developing NAFLD. In 2008, a study scanned the genomes of participants in a large population-based study to uncover a variant in a gene called *PNPLA3* that was strongly associated with NAFLD and was more common among Hispanic individuals with higher liver fat and inflammation. In 2010, studies of genomic data from participants in the NASH Clinical Research Network, as well as patients with NASH studied at the NIH Clinical Center, found that the *PNPLA3* variant was also associated with earlier disease development in children. These and other recent studies have identified additional regions of the genome associated with NAFLD, including a gene known as *APOC3*.

STORY OF DISCOVERY

More to Discover

In the future, NIDDK anticipates new discoveries from the NASH Clinical Research Network and efforts by individual investigators. In these ways, NIDDK will continue its efforts toward preventing and treating the once "unnamed" disease of NASH, as its name becomes increasingly familiar.

Bacteria and the Immune System Work Together To Peacefully Coexist in the Intestine

Dr. Charles O. Elson III

Dr. Charles O. Elson III is Professor of Medicine and Microbiology and the Basil I. Hirschowitz Chair in Gastroenterology at the University of Alabama at Birmingham. Dr. Elson received his M.D. from Washington University in St. Louis, and he completed a residency in internal medicine at Cornell, an NIH fellowship in gastroenterology at the University of Chicago, and a postdoctoral fellowship in the Metabolism Branch at the National Cancer Institute. Dr. Elson is an elected member of the Association of American Physicians, an elected Fellow of the American College of Microbiology, former President of the Society for Mucosal Immunology, and has served on the National Diabetes and Digestive and Kidney Diseases Advisory Council. His research, which has been supported by the NIDDK, has made seminal contributions to understanding the regulation of immune responses in the digestive tract. At the February 2010 Advisory Council meeting, Dr. Elson presented advances from his research on the cellular and molecular mechanisms controlling the immune response to bacteria in the intestine; the following are highlights from his presentation.

The Intestinal Microbiota

The human intestine is host to an enormous ecosystem of microorganisms. This microbial community—or microbiota—is very complex. And, with a population of nearly 100 trillion organisms, the number of bacterial cells in the intestine outnumbers human cells by almost 10 to 1. While the presence of intestinal microbes has been appreciated for over a century, there is growing interest in understanding the composition of the microbiota and its role in shaping human health and disease. Recent advances—through initiatives such as the Human Microbiome Project—have started to define the diversity of bacterial species and bacterial genes present in the intestines. This has revealed aspects of the microbial community's metabolic functions and its influence on the development of the intestinal immune system.

The microbiota is established in the intestine shortly after birth. Humans and other animals tend to live in harmony with their gut microbes throughout their life. Given how intimate this relationship is, researchers have puzzled over what allows us—humans and our microbes—to peacefully coexist. Why doesn't the immune system normally attack this mass of resident microbes encountered in the intestines? How does the intestine maintain the balance, or "homeostasis," between friend and foe?

Intestinal Homeostasis in Mice and Men

Dr. Elson and his research group have studied human intestinal biology and disease using mice as an informative model. In particular, Dr. Elson's team has studied a strain of mice, called C3H/HeJBir, that spontaneously develops inflammation of the colon (colitis). They discovered that this mouse has a strong immune response to some component of the microbiota. To learn more about this immune response, the researchers focused on the role of T cells, a type of immune cell that recognizes and responds to specific molecules, such as bacterial components. Remarkably, when they isolated T cells from C3H/HeJBir mice with colitis and transferred these T cells to other mice lacking their own immune cells, the recipient mice developed colitis.

Dr. Elson next wondered what parts of the bacteria in the intestine might be activating these T cells

to generate the aberrant immune response that causes colitis. Using a molecular screening technique, Dr. Elson's team identified a number of T cell-activating bacterial components, and many turned out to be proteins known as flagellins. Flagellins make up the tail-like structures, called flagella, that some bacteria use to move.

Based on these findings, the scientists decided to test more directly whether T cells reactive to a bacterial flagellin can cause intestinal inflammation. To do this, they generated T cells that respond to one specific type of flagellin called CBir1. When they transferred these CBir1-specific T cells into other mice (which were deficient in their own immune system), the mice developed colitis. Interestingly, T cells that were artificially activated (*i.e.,* not in response to the bacterial flagellin) did not cause intestinal damage. These results supported the idea that an abnormal immune response to specific components of the microbiota causes intestinal inflammation.

In a collaboration facilitated by his NIDDK Program Project grant, Dr. Elson and his colleagues then partnered with other scientists to translate this exciting result very rapidly into understanding of human disease. They found that patients with Crohn's disease—a form of inflammatory bowel disease (IBD)—had elevated levels of antibodies specific for this bacterial flagellin. Antibodies are produced by another type of immune system cell. In addition, the presence of these antibodies was predictive of a more complicated disease course for these people. This was an exciting example of how basic research discoveries made in animal models can be translated to advance knowledge of human disease.

Interestingly, results from a very different line of research—human gene mapping—are also starting to converge with the T cell experiments in mice. Dr. Elson pointed out that in genomic studies of IBD, scientists have identified human genetic variants that are associated with immune system function in a way that's related to the inflammatory reaction caused by T cells in the mouse model. This is an exciting example of how results from animal models complement studies of humans to enhance our understanding of disease.

Suppressing an Adverse Immune Response to Gut Bacteria—the Role of Immunoglobulin A

Having found that this very potent bacterial trigger of inflammation is present in the intestine and associated with disease course in humans, Dr. Elson was interested in understanding how the intestinal immune system normally deals with its presence—what keeps T cells from causing an inflammatory response to this ever-present bacterial structure in a healthy intestine?

To address this question, Dr. Elson created a mouse model whose T cells are specific for only the CBir1 flagellin. Much like the C3H/HeJBir mouse strain, it was expected that a mouse with T cells specific for CBir1 would also develop colitis. Surprisingly, these mice turned out to be healthy. Their intestinal tissues were normal, and they did not have the expected elevated levels of flagellin-specific antibodies in their blood. Something was preventing the CBir1-specific T cells from becoming activated.

Further investigation uncovered new pieces to this puzzle. Although the mice did not have antibodies to CBir1 flagellin in their blood, Dr. Elson found that the mice had very high levels of a different type of CBir1-specific antibody in their intestines. There are several different types of antibodies. The most common type in blood is called immunoglobulin G (IgG). The most abundant type of antibody in the intestine, on the other hand, is immunoglobulin A (IgA). Dr. Elson's team found that the mouse model with CBir1-specific T cells had a large amount of intestinal IgA specific for the CBir1 flagellin. Interestingly, they found that normal mice also had a very high level of CBir1-specific IgA in their intestines. (In their previous experiment, the T cells from C3H/HeJBir mice caused colitis in mice lacking an immune system, as those mice also lack IgA.)

These findings suggested that IgA may play an important role in the normal response to gut microbes. Dr. Elson's team designed an elegant series of experiments to determine what precisely the CBir1-specific IgA was doing in the intestine. First, they isolated the CBir1-specific T cells from their mice and labeled the T cells with a dye. This dye allowed them to monitor when the T cells become activated. They then transferred the labeled CBir1-specific T cells back into different mice, and fed the mice CBir1, thinking that a large amount of this bacterial flagellin introduced into their gut might activate the T cells. When they transferred the T cells into normal mice and fed them CBir1 flagellin, the T cells remained inactive. It turned out that if they transferred the T cells into mutant mice that do not produce any IgA, however, the T cells were robustly activated by orally administered CBir1 flagellin. These results suggested that, under normal conditions, IgA in the intestine prevents this bacterial flagellin from activating its cognate T cells.

Preventing Destructive Immune Responses—A Molecular Team Effort

Dr. Elson's experiments established that IgA is important in restricting the T cell response to flagellin from the intestinal microbiota. However, there are different types of T cells with very different functions in the body. Previous studies have shown that a type of T cell known as regulatory T cells (Tregs) are abundant in the tissue lining the intestine and that they are involved in inhibiting immune responses. Unlike the T cells that are activated by bacteria to promote inflammation, Tregs produce a factor called TGF-beta, which in addition to inhibiting immune responses, also turns on production of IgA by antibody-producing cells. This raised the question as to whether IgA and Tregs operate independently or if somehow they cooperate to suppress the immune response to resident gut bacteria.

In another elegant series of experiments, Dr. Elson and his research team demonstrated that Tregs are, in fact, important in inducing the IgA response to CBir1 flagellin. In their study, the researchers used an experimental technique to reduce the number of Tregs in otherwise normal mice, and then assessed whether there was a resulting effect on IgA levels. When they depleted the levels of Tregs, the scientists found that the total amount and CBir1-specific amount of IgA dropped to negligible levels compared to mice that have normal levels of Tregs. From the results of these and related experiments, Dr. Elson and his team concluded that Tregs are indeed important for inducing the IgA response to this specific microbial product.

Gut Microbes and the Immune System Work Together To Prevent Infection

In addition to shedding light on how adverse immune reactions are prevented against normal gut microbes, Dr. Elson's research also suggests how IgA, Tregs, and the microbiota work together to help prevent infection by invading bacteria.

It is well known that in addition to its metabolic properties, the normal resident gut microbiota helps prevent infection in the intestine. That is to say, the presence of the microbiota itself makes it difficult for potentially pathogenic bacteria to take up residence and cause infection. However, it might not be expected that the combination of Tregs and IgA would be beneficial to fighting pathogenic bacteria, because Tregs also shut off the immune response that could help stave off infection.

Dr. Elson explained that this apparent paradox is reconciled when considering IgA and Tregs as working in concert with the microbiota. The IgA molecules provide a protective layer on the surface of cells lining the intestine. In this position, IgA can bind to flagellins—or other bacterial structures—from the normal microbiota and provide a foothold for the resident bacteria to live in the intestine and carry out their metabolic functions. Since Tregs are also responsive to these normal gut bacteria, they provide

a second line of defense by stimulating production of IgA and suppressing a potentially harmful inflammatory response. This further stabilizes the microbiota and helps to maintain resistance against invasion by pathogenic bacteria.

These findings have important implications for the development of oral vaccines targeting immune responses in the gastrointestinal tract. In addition to existing vaccines that are typically given by injection and generate antibodies in the bloodstream, scientists have also tried to develop vaccines that could be administered orally to protect against infectious bacteria. Some of the challenges that have been encountered in developing oral vaccines could be explained by Dr. Elson's research. That is, the mechanisms that normally prevent inflammatory responses against resident gut bacteria may also be restraining the intestinal immune system from mounting a lasting protective reaction to oral vaccines.

Conclusions

In summary, Dr. Elson's research is illuminating how animals and their resident intestinal microbes peacefully live together. The results he presented demonstrate a regulatory role of IgA in maintaining intestinal homeostasis. In addition, he showed that Tregs, which are abundant in the tissue lining the intestine, work in a coordinated fashion with IgA and the microbiota to maintain homeostasis and restrict an adverse, inflammatory immune response. Various aspects of Dr. Elson's discoveries in mouse models of intestinal inflammation have already been translated to inform our understanding of IBD in humans. These studies point to the biological pathways regulating the immune response to intestinal bacteria as being important in human health and disease.

PATIENT PROFILE

David Warren

Nonalcoholic Steatohepatitis (NASH) Study Finds Promising Treatments for Hidden Liver Disease

David Warren

A few years ago, 62-year-old David Warren was loving life. Retired for 3 years after 32 years of working for the U.S. Postal Service and happily married, David went about life doing what he likes best—gardening and playing the stock market from his home computer.

However, after a routine annual checkup, David's blood work showed that his liver enzymes were elevated, a sign of injury or disease in the liver. "I'd been taking a statin [a drug that can sometimes elevate liver enzymes] to help control my cholesterol, and so my primary care physician sent me to Duke University Hospital for a liver biopsy to find out whether taking the statin was the cause of my elevated enzyme count," says David.

When his biopsy report came back, David learned that something else was causing his liver damage. It turned out that he had a form of liver disease called nonalcoholic steatohepatitis (NASH), which is characterized by excess fat and inflammation in the liver. If left undiagnosed and untreated, over time NASH can lead to liver failure—requiring a liver transplant—or to the development of hepatocellular carcinoma, a form of liver cancer.

When they received the diagnosis, David and his wife were both shocked and scared. "We couldn't believe it," says David. "We always associated liver disease with drinking—and I don't drink."

Immediately after receiving his NASH diagnosis, David was asked if he'd be willing to participate in a clinical trial called "PIVENS." Supported by NIDDK, with additional support from private industry, PIVENS is a trial within NIDDK's NASH Clinical Research Network, which was established to study the natural history, disease processes, and therapy of NASH in both adults and children. The PIVENS trial tests treatments for NASH in adults, while another clinical trial, called "TONIC," focuses on treating NASH in children.

David readily agreed to participate in the PIVENS trial.

When they received the diagnosis, David and his wife were both shocked and scared. "We always associated liver disease with drinking—and I don't drink."

About NASH

Recognized as a specific medical condition in 1980, NASH is believed to be on the rise in the U.S., most likely, researchers say, as a result of the epidemic

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increase in obesity. Although most people with NASH, like David, do not manifest symptoms for several years, a great danger of this disease is that it can lead to a cirrhotic liver, a condition in which the liver develops extensive scar tissue that stiffens blood vessels and distorts the internal structure of the liver. Over time, NASH can lead to significant scarring of the liver and liver cirrhosis. Liver cirrhosis may progress to liver failure, ultimately requiring liver transplantation. Currently, there are no specific Food and Drug Administration-approved treatments available for NASH.

Although the specific cause or causes of NASH are unknown, in addition to its link with obesity, the disease also is typically associated with insulin resistance or even type 2 diabetes, hypertension, elevated lipids, or other features of metabolic syndrome. Oxidative stress—a state which produces chemicals called free radicals that can cause damage to the body's proteins, membranes, and genes—also is thought to play a role.

At the time of his NASH diagnosis, David had many of the common risk factors for developing the disease, including high blood pressure, high cholesterol, high triglycerides, and insulin resistance.

"I've been insulin resistant, or pre-diabetic, for probably 4 or 5 years," says David. "My mother had diabetes, and I'm pretty sure my father did, too," he adds. Never morbidly obese, David was aware, however, that he was carrying too much weight and that he could afford to lose a few pounds.

Therefore, even prior to his NASH diagnosis, and concerned about his insulin-resistant condition turning into full-blown type 2 diabetes, David began exercising and watching his diet. "I use an elliptical rider and a recumbent bike, as well as work in my flower garden 2 or 3 hours a day," he says. It may have been enough to tip the scales in his favor.

The PIVENS Trial

PIVENS is short for Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis. With enrollment starting in 2005, PIVENS was conducted at eight clinical centers around the country participating in the NASH Clinical Research Network, including the Duke University center where David participated in the trial, and a data coordinating center. It is the largest randomized, placebo-controlled clinical trial of NASH therapy to date.

As its name implies, PIVENS was designed to study whether the antioxidant vitamin E or the drug pioglitazone, which improves insulin sensitivity in cells, could be used to treat adults with NASH, like David, who were not currently or previously taking an insulin-sensitizing drug and who don't currently have diabetes.

Over a 2-year period, David and other PIVENS volunteers were given once-daily doses of either pioglitazone, vitamin E, or a placebo. During the trial, participants did not know which agent they were taking. Liver biopsies were taken before and after the trial to reveal whether these agents improved signs of NASH in the liver, the main focus of the trial. Other information collected on participants included measures of liver health and metabolic fitness, as well as any side effects from these agents.

In May 2010, results of the PIVENS trial were announced in the *New England Journal of Medicine*, showing that vitamin E significantly improved NASH in adults based on a constellation of disease features, including liver inflammation, fibrosis, and fat accumulation. Although pioglitazone did not significantly reduce this overall measure of NASH in the liver, it showed some positive effects, including more normalized liver enzymes and reduced fat in the liver.

To David's and his wife's great relief, as the months of his participation in the study went by, his NASH

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did not progress. By the end of the study, a biopsy of his liver no longer showed the changes characteristic of NASH. "The disease is well under control, and I'm no longer taking any medication for it," says David.

"I can't see why people wouldn't volunteer for these studies," says David. "It's a win-win for everybody. It changed my life."

Since the completion of the treatment phase of the study, David has continued to participate in the 6-month follow-up phase. He learned that he had been taking pioglitazone every day during the treatment phase of the study, and the only side effect he experienced was weight gain, which was also observed in other study participants taking the drug.

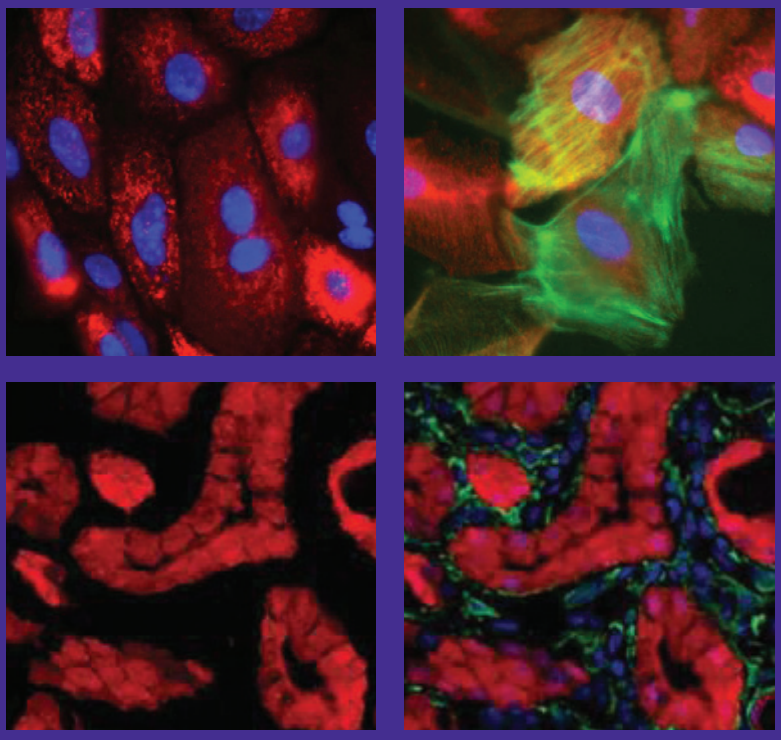
After the treatment phase (discontinuing the drug), through exercise and a healthy diet, David has lost 18 pounds, "but my family physician said I should lose at least another five," he says.

An added benefit of David's participation in the clinical trial was that doctors were able to discover that he had severe sleep apnea—and thus start him on a treatment for this condition from which he had been suffering for years. Untreated, sleep apnea can lead to heart attack, heart failure, irregular heartbeats, and diabetes. "For years I would wake up gasping for breath, but wasn't quite sure what was wrong with me," says David. Now he sleeps with an air mask, and reports sleeping very well at night.

"I can't see why people *wouldn't* volunteer for these studies," says David. "It's a win-win for everybody. It changed my life."

David is now loving life again. He enjoys working in his garden more than ever, and continues to adapt to whatever comes his way. "I used to plant vegetables, but the deer seem to like them more than I do," he laughs.

For additional info on the PIVENS trial and other NASH research efforts, please see related content in the research advance section and Story of Discovery in this chapter.



Mapping where cells move and how they act after injury can provide important information about the mechanisms of kidney injury and repair. New work has identified methods to unambiguously mark cells throughout their life. In culture, mouse kidney tubule cells (red, top left) can be transformed into primitive mesenchymal cells (green and yellow, top right). But in the intact mouse, this transformation does not occur, and injury does not cause tubule cells (red) to move out of the tubule and transform into primitive mesenchymal cells (green, bottom left). This research identified novel targets for treatment of kidney injury. For more information on this topic, please see the corresponding research advance in this chapter.

Images provided by Dr. Jeremy S. Duffield and reproduced with permission of the American Society for Investigative Pathology, from Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis, Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, and Duffield, JS, American Journal of Pathology, volume 176, edition number 1, 2010; permission conveyed through Copyright Clearance Center, Inc.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. They afflict millions of Americans, including children and young adults. The NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The goal is to increase understanding of kidney, urologic, and hematologic diseases to enhance prevention and treatment strategies.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, even for a short period of time or due to gradual deterioration, can result in life-threatening complications. Whether kidney function is lost suddenly or slowly represents an important health challenge.

Chronic kidney disease has two main causes: high blood pressure and diabetes. Recent estimates put the number of Americans with chronic kidney disease at more than 23 million.[1] If unchecked, the recent increases in obesity and type 2 diabetes in the U.S.—especially among children and adolescents—have grave implications, as individuals are likely to face any secondary health consequences at an earlier age than people who develop these conditions as middle-aged adults.

Chronic kidney disease, 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. At the close of 2008, nearly 550,000 patients were receiving treatment for ESRD: over 380,000 were undergoing dialysis and over 165,000 were living with a kidney transplant. Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease and ESRD. African Americans are nearly four times more likely to develop kidney failure as non-Hispanic whites. American Indians and Hispanics have twice the risk for kidney failure as do non-Hispanic whites.[2]

The NIDDK supports a significant body of research aimed at understanding the biology underlying chronic kidney disease. The Institute'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 treatments to prevent development or halt progression of kidney disease. Also of interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome. The Institute'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 chronic kidney disease and prevent kidney failure. It represents a major educational outreach effort to patients, physicians, and the public. In October 2010, NKDEP hosted a meeting titled "Translating Chronic Kidney Disease Research into Improved Clinical Outcomes." It focused on research to identify factors that lead to adoption, maintenance, and sustainability of science-based interventions in real-world clinical settings.

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 Program supports basic and clinical research on the normal and abnormal development, structure, and function of the genitourinary tract. Areas of particular interest include the causes of and treatments for major adult urological diseases and disorders, such

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

[2] *U.S. Renal Data System, USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the U.S., National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2010.*

as benign prostatic hyperplasia, urinary incontinence and urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/chronic pelvic pain syndrome in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating, chronic, and painful urologic disorder. IC/PBS affects both men and women, but it is nine times more common in women. NIDDK-supported basic and clinical research is focused on elucidating the causes of IC/PBS, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. The NIDDK's Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network supports studies designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. The goals and approaches of the MAPP Research Network reflect the most current thinking on IC/PBS pathology and involve significant new advancements in how IC/PBS is studied. All efforts are designed to provide insights that can be translated to improve the clinical care of patients with IC/PBS. A prospective epidemiological study in a racially and ethnically diverse sample of men and women, the Boston Area Community Health Survey (BACH), seeks to identify patterns and risk factors for those bothersome symptoms. A similar study, the Olmsted County (Minnesota) Study, is studying lower urinary tract symptoms in men.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.[3,4] Many suffer in silence due to embarrassment and lack of knowledge about options available. The introduction of new surgical procedures has advanced the treatment of urinary incontinence dramatically in the last decade. The NIDDK's Urinary Incontinence Treatment Network recently completed the Trial of Mid-Urethral Slings showing that the two most common mid-urethral sling procedures are similar in their chance of cure for stress urinary incontinence, though each surgery has different risks. Additional information regarding the study can be found later in this chapter.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. The Institute is also keenly interested in the basic biology of stem cells, including adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

The year 2010 marked the 100th anniversary of the first detailed case report of sickle cell disease. In the U.S., between 70,000 and 100,000 people are affected with this disease, mainly individuals of African ancestry. In November 2010, NIH held the "James B. Herrick Symposium—Sickle Cell Disease Care and Research: Past, Present and Future." At the symposium, which was named after the physician who first described the disease, national and international experts in sickle cell care and research discussed the history and societal impact of the disease along with current and future basic, translational and clinical research. The NIDDK Director Dr. Griffin Rodgers, a renowned sickle cell disease researcher, was a featured speaker.

GENETICS OF KIDNEY DISEASE

New Genetic Regions Found To Be Associated with Kidney Function and Chronic Kidney Disease: Researchers have identified 20 new genetic regions in which variants seem to be associated with

[3] Nygaard I, et al: Urinary Incontinence in Women in Urological Diseases in America (pp. 157-191). NIDDK, NIH Publication Number 07-5512, 2007.

[4] Stothers L, et al: Urinary Incontinence in Men in Urological Diseases in America (pp. 193-221). NIDDK, NIH Publication Number 07-5512, 2007.

increased susceptibility to chronic kidney disease. Members of the CKDGen consortium analyzed data from genome-wide association studies of over 67,000 people to try to identify alternate genetic sequences that were correlated with either diagnosed chronic kidney disease or evidence of reduced kidney function. The researchers assessed reduced kidney function based on levels of the molecules creatinine and cystatin c circulating in the blood. These molecules are usually filtered out of the blood by the kidneys; thus, the circulating level of these molecules can be used to estimate kidney function, and their levels tend to rise as kidney function declines.

Once candidate genetic regions were identified in the initial analysis, they were tested again using a second set of samples from nearly 23,000 additional people. This two-step approach identified 13 genetic regions that appear to be related to kidney function and chronic kidney disease, and an additional 7 that are thought to be involved in creatinine production and secretion. The regions identified by these analyses are believed to include genes related to kidney development, filtration, transport of small molecules and salts, and other metabolic functions of the kidneys. The identification of multiple common genetic variants that seem to be associated with various aspects of kidney function and kidney disease furthers scientists' understanding of the basic biology underlying kidney development and function. These findings also may help explain some of the marked variability in the likelihood of developing kidney disease among people with diabetes and high blood pressure, the two most common causes of kidney disease and kidney failure.

Köttgen A, Pattaro C, Böger CA, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet 42: 376-384, 2010.

Gene Variants that May Protect Against Parasitic Disease also Lead to Increased Risk of Kidney Disease: Researchers have found that variants in the *APOL1* gene that are more common in African Americans come with both health benefits and risks. On one hand, they provide protection from African sleeping sickness, but on the other, they confer an increased likelihood of developing kidney disease. In 2008, researchers reported that genetic variations on chromosome 22 were linked to greater incidence of non-diabetic kidney disease among African Americans. Although these variations were at first thought to be related to the *MYH9* gene, researchers have now found that much of the increased risk of kidney disease is due to two specific variations in the adjacent *APOL1* gene. This gene encodes a circulating protein that, in its mutant form, has been shown in experiments to destroy trypanosomes, which are parasites that carry African sleeping sickness, a degenerative and potentially fatal disease affecting tens of thousands of people in sub-Saharan Africa. These two *APOL1* variants appear to have evolved relatively recently—in the past 10,000 years or so. Their relatively recent appearance and frequency in chromosomes in individuals of African descent suggests that the protection these variants provide against parasitic infection is significant.

It is currently unclear what the precise biological function of the protein encoded by this gene is, nor is it clear how these mutations might contribute to kidney disease. Given the high frequency of these *APOL1* variants in people of African descent and their strong effect on kidney disease risk, unraveling the molecular mechanisms by which they contribute to kidney injury could provide important insights into the causes of and possible treatments for kidney disease in African Americans.

Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science 329: 841-845, 2010.

New, Improved Genetic Screening Method Identifies a Novel Genetic Cause of a Rare Form of Kidney Disease: Researchers have recently used a novel, faster genetic screening approach to identify a gene related to the development of a rare but devastating form of kidney disease. Nephronophthisis-related ciliopathies (NPHP-RC) are genetic cystic disorders that manifest in the kidney, eye, brain, and liver and lead to tissue degeneration. Collectively, nephronophthisis is the most common genetic cause of end-stage renal failure in people under age 30. Mutations in nine genes are known to result in NPHP-RC, but these appear to account for less than 1 percent of all cases. The identification of additional genetic contributors to disease has been

hampered by the fact that these diseases are very rare, often appearing in single families, and because standard techniques for identifying candidate genes return too many potential genetic variants to be able to narrow down the possibilities with only a small number of samples.

The researchers combined two different genetic screening approaches to advance their search for disease genes. One of these, called "exome capture," examines just that portion of the genome that codes for proteins. The other approach is based on the knowledge that these rare kidney diseases tend to occur when a person inherits the same gene variant from each parent, such that both of the person's copies of the gene are mutated. With this combined strategy and advanced technology for determining DNA sequences across genomes, researchers identified 12 new mutations in the *SDCCAG8* gene that were associated with NPHP-RC in 10 families. Characterization of the protein encoded by this gene showed that it is associated with the centrioles, barrel-shaped structures within the cell that are involved in cell division as well as in the formation of cilia, which are tiny, hair-like projections on the surface of many cells. Cilia collect information about the cells' environment, and defects in cilia and their signaling properties have been shown to play a role in several diseases. The SDCCAG8 protein was also found to interact with another protein that previously had been shown to be associated with the development of NPHP-RC. Studies in zebrafish—an important vertebrate model organism in scientific research—showed that depletion of the SDCCAG8 protein led to defects in body axis development and the formation of cysts in the kidneys. Moreover, cultured kidney cells in which SDCCAG8 protein had been depleted were unable to form higher-order structures, indicating a defect in cell polarity and tubule formation.

These results strongly suggest that loss of SDCCAG8 function can cause NPHP-RC, possibly by disrupting the ability of the cell to sense its orientation in three-dimensional space. They also validate exome capture in combination with other targeted genetic screening approaches as an experimental strategy for identifying candidate mutations in genetic disease. This approach may help speed the search for the causes of many other single-gene disorders. It may also facilitate the search for agents to treat them, as it would allow the screening of large numbers of compounds that may halt disease initiation or progression.

Otto EA, Hurd TW, Airik R, et al. Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. Nat Genet 42: 840-850, 2010.

SLOWING THE PROGRESSION OF CHRONIC KIDNEY DISEASE

Lower Blood Pressure Goal Benefits African Americans with Chronic Kidney Disease and Protein in the Urine: The latest results from a long-term study show that, on average, a lower blood pressure goal was no better than the standard blood pressure goal at slowing progression of kidney disease among African Americans who had chronic kidney disease (CKD) resulting from high blood pressure. However, the blood pressure goal did benefit patients who had protein in the urine, a sign of kidney damage. This same trial also found that among people with protein in their urine, keeping blood pressure at the lower level reduced the likelihood of kidney disease progression, kidney failure, or death by 27 percent compared to the standard blood pressure level—a statistically significant difference. These results come from the African American Study of Kidney Disease and Hypertension (AASK), the largest and longest study of CKD in African Americans.

In the U.S., high blood pressure causes about one third of new cases of kidney failure. The AASK trial has followed participants for approximately 12 years to measure the long-term effects of blood pressure control in African Americans with kidney disease attributed to high blood pressure. In its initial phase, the AASK study found that a drug that targeted the renin-angiotensin system, specifically an ACE inhibitor, was more effective at slowing the progression of kidney disease in African Americans than other classes of drugs. A subsequent follow-up study found that kidney disease worsened in about one-quarter of study participants in spite of the best available treatment, while another one-third of participants experienced only a slow decline in kidney function, about what is generally observed with aging.

This most recent finding that, in some patients, more intensive control of blood pressure may slow progression of chronic kidney disease adds important new information about which patients with kidney disease may benefit from lowering of blood pressure beyond the standard goal. It may help doctors practice evidence-based, personalized medicine, tailoring the treatment regimen to each patient's unique characteristics. This study also highlights the importance of conducting long-term clinical studies, because without the follow-up study, the benefits of the lower blood pressure goal in a subset of patients with protein in their urine might have been missed.

Appel LJ, Wright JT, Greene T, et al. Intensive blood-pressure control in hypertensive chronic kidney disease. N Engl J Med 363: 918-929, 2010.

KIDNEY FIBROSIS RESEARCH

Identification of the Cellular Source of Scar-Producing Collagen in a Model of Kidney Fibrosis: Scientists have recently pinpointed a type of cell in the kidney that appears to be a source of much of the scar tissue that is seen in some forms of kidney disease. "Fibrosis" is the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to scarring within an organ. It is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis can impair the removal of toxins and excess fluid from the blood, cause irreversible kidney damage and, in extreme cases, lead to kidney failure.

The origin of the collagen that comprises the fibrotic scar in kidney disease has remained a mystery. Many scientists have hypothesized that kidney fibrosis may be mediated, at least in part, by cells that migrate out of the kidney tubule into surrounding tissue and begin secreting collagen. This speculation stems from studies using cells grown in culture dishes in which kidney cells can become collagen-producing cells called myofibroblasts, a phenomenon referred to as "epithelial-to-mesenchymal transformation." To investigate whether this was occurring in whole kidneys, researchers genetically manipulated mice so that specific subtypes of cells in their kidneys contained an easily detectable molecule, or "label." They surgically induced fibrotic kidney disease in these animals and, after about 2 weeks, examined the kidneys for scar formation. Contrary to prevailing theories, the labeled cells in the kidney tubule did not migrate into the surrounding tissue and participate in scar formation. Instead, myofibroblasts that were already present in the kidney seemed to be the source of collagen. Myofibroblasts are derived from pericytes, a type of stem cell that is usually associated with blood vessels.

This study provides strong evidence that kidney tubule cells do not migrate or undergo epithelial-to-mesenchymal transformation in kidney fibrosis. Rather, kidney fibrosis appears to arise through a novel pathway involving pericyte-derived myofibroblasts. A more complete and accurate understanding of collagen deposition and scar formation is a key first step in devising novel therapies aimed at preventing kidney fibrosis. These studies illustrate the value of animal models in providing important insights into biological processes. They also suggest that therapeutic approaches targeting pericytes may prove beneficial in patients with fibrotic kidney disease.

Humphreys BD, Lin S-L, Kobayashi A, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol 176: 85-97, 2010.

Scientists Identify a Potent Inhibitor of Kidney Fibrosis: Researchers have identified the circulating protein serum amyloid P (SAP) as a natural inhibitor of fibrosis during inflammatory injury in the kidney. Using two different models of kidney injury and fibrosis in mice, researchers found that human SAP can potently inhibit fibrosis in this organ. SAP accumulated at sites of injury within the kidney, where it appeared to be associated with injured or dead cells. In the kidney, SAP acts on monocytes and macrophages, specialized white blood cells that are involved in the inflammatory response. SAP suppresses the activity of these inflammatory cells by binding to Fc-gamma receptors on their surfaces. This inhibition of cell activity is dependent on the increased production of the anti-inflammatory protein interleukin-10. SAP has previously been shown to suppress fibrosis in the lung, but through a different mechanism than that seen in the kidney. Taken together, these observations suggest that SAP may have the potential to act as a broad-based anti-fibrotic agent.

The repair of organ and tissue damage is a complex and multistep process, with an initial inflammatory response that attempts to resolve the insult coupled with wound healing and tissue remodeling. Under certain conditions, unrestrained tissue "repair" leads to the excessive deposits of fibrous scar tissue. This process, termed fibrosis, can impair organ function and, left unchecked, lead to organ failure.

This research identifies previously unknown mechanisms of action of SAP in regulating anti-inflammatory activity, and raises the possibility of using SAP or similar agents as a therapy for fibrotic kidney diseases. Subsequent testing in patients who have ongoing fibrotic diseases may determine whether the therapeutic potential seen in these mouse studies can be translated into a novel clinical intervention in patients.

Castaño AP, Lin S-L, Surowy T, et al. Serum amyloid P inhibits fibrosis through Fc-gamma R-dependent monocyte-macrophage regulation in vivo. *Sci Transl Med 1: 5ra13, 2009.*

AUTOIMMUNE KIDNEY DISEASE

New Insights into the Immunology of Goodpasture's Syndrome: Scientists have recently reported new findings on the immunological targets relevant to a debilitating autoimmune disease known as Goodpasture's syndrome. This disease is marked by kidney damage, often leading to kidney failure, and bleeding in the lungs. Previous studies of Goodpasture's syndrome have suggested that the body's immune system mounts a misguided antibody attack that targets the collagen networks found within the kidney. These networks are composed of bundles of rope-like collagen molecules that are linked together in a way that, among other functions, helps provide support for surrounding cells. In Goodpasture's syndrome, it has been postulated that a change in the shape of one of the collagen subunits exposed a normally hidden region of the molecule, triggering the immune response. Indeed, patients with Goodpasture's syndrome have circulating antibodies against a region of one of the collagen strands, but details regarding the actual target region(s) have remained elusive.

The new study identifies two specific anti-collagen antibodies in the kidneys and lungs of patients with Goodpasture's syndrome that react with two distinct sites on the collagen strand. The presence of the antibodies in these two tissues provides strong evidence that they play a role in disease progression. Using this knowledge, researchers may be able to design "decoy" targets for the self-reactive antibodies that could either prevent them from binding collagen molecules in the kidney or otherwise remove them from the circulation. An important issue to be clarified in future studies is determining what events lead to the initial shape change of the collagen molecule and whether this in fact provokes the immune response or whether some other, yet unknown, factor is responsible for both events.

Pedchenko V, Bondar O, Fogo AB, et al. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. N Engl J Med 363: 343-354, 2010.

KIDNEY REPAIR

New Component of the Kidney Repair Machinery Characterized: Investigators studying a rat model of kidney injury recently identified a new component of the injury repair machinery. This finding has important implications because there is relatively little known about normal repair mechanisms, and new knowledge could provide targets for novel therapeutic strategies.

In normal mice, a protein called Gpnmb is highly expressed in lung, pancreas, and skin, but very little is found in the kidney. Using rodent models of kidney injury in which the blood supply to the kidney is temporarily restricted, scientists discovered that Gpnmb levels markedly increased during the repair phase after blood flow was restored. Gpnmb levels increased 15-fold in kidney tissue, more than 10-fold in an immune cell type called macrophages that were in the kidney, and 3-fold in surviving kidney epithelial cells, which help carry out many kidney functions. Gpnmb levels were higher in regions of the kidney that suffered more severe injury than in areas that were less seriously damaged.

Macrophages—the name means "big eater"—are a specialized type of immune cell that engulf and digest cellular debris and foreign bodies, and this process is an important part of recovery from injury. Recent studies have suggested that injured epithelial cells

might also play an important role in the clearance of debris and dying cells through a similar mechanism during tissue repair. To examine the possible connection between increased Gpnmb expression in both macrophages and kidney epithelial cells, these cell types were microscopically examined for the presence of "apoptotic bodies," the remnants of dead cells. Both macrophages and epithelial cells containing Gpnmb were found to have more apoptotic bodies than their counterparts deficient in Gpnmb.

Kidney tissue repair following experimentally induced ischemia (restriction of blood supply) was next evaluated in normal mice and in mice lacking Gpnmb. Gpnmb-deficient mice were found to have a five-fold increase in the number of dead cells in their kidney tissue compared to control animals, providing evidence that Gpnmb plays an important role in either cell death or in the removal of dead cells by macrophages and epithelial cells. Moreover, in an animal model in which macrophages had been depleted, recovery from injury was delayed compared to animals containing normal levels of macrophages.

Taken together, these results provide strong evidence that Gpnmb levels are increased in the kidney during injury repair, and that it promotes repair by facilitating the degradation of debris by both macrophages and epithelial cells. This finding is notable because these two cell types were not previously known to work collectively in the tissue repair process, information that may contribute to the development of new treatments for kidney injury.

Li B, Castano AP, Hudson TE, et al. The melanoma-associated transmembrane glycoprotein Gpnmb controls trafficking of cellular debris for degradation and is essential for tissue repair. FASEB J 24: 4767-4781, 2010.

UROLOGY RESEARCH

The Role of Bacterial "Capsules" in Urinary Tract Infections: The presence of a particular kind of capsule is required for infectious bacteria to grow and form large masses called intracellular bacterial communities (IBCs) within the urinary tract, according to a recent report. Infections of the urinary tract are common in women—about one-third of all women in the U.S. are diagnosed with a urinary tract infection (UTI) by the time they reach 24 years of age—and many women suffer repeated UTIs. While antibiotic treatments are available, better prevention and treatment strategies are needed. Most UTIs are caused by a common type of *Escherichia coli* (*E. coli*) bacterium. An acute UTI begins when bacteria attach to cells lining the inside of the bladder. This provokes a defense response in the infected individual, including activation of the immune system and sloughing off of bladder cells into the urine in an attempt to rid the body of bacteria. To escape the host defense response, UTI-causing *E. coli* are able to invade cells lining the bladder and form IBCs. After an IBC has grown extensively within an infected bladder cell, the bacterial community exits the infected cell and invades uninfected cells, establishing a cyclical pattern of infectivity.

How are IBCs so effective in evading the host defense response? To address this issue, scientists hypothesized that the K1 capsule that surrounds *E. coli* is involved in the bacteria's ability to form IBCs. Encapsulation is a well-established feature of bacteria that causes disease; for example, the K1 type of capsule has been shown to play a key role in the ability of bacteria to cause meningitis in the rat. Composed of molecules of sialic acid (a complex chain of sugar molecules), the K1 capsule has been shown to inhibit the activity of immune system cells called neutrophils.

In this study, scientists generated *E. coli* strains containing different mutations of the K1 capsule and compared these to normal UTI-causing bacteria to determine whether the capsule plays a role in various stages of UTI disease development in mice. Compared to non-mutated *E. coli*, K1 capsule mutants were found to be less efficient in different stages of disease development, including the formation of IBCs, cell growth, and prevention of neutrophil infiltration into infected bladders. The scientists also found that a specific K1 capsule mutation that results in accumulation of sialic acid within the bacterium has an even more significant negative effect on IBC formation, likely *via* sialic acid's putative role in controlling the levels of various proteins involved in the development of UTIs. By identifying the bacterial capsule as a factor that contributes to IBC formation, the scientists have found targets for potential novel therapeutic interventions to prevent or treat UTIs. As IBCs have

been observed in human bladder infections, these results likely have direct clinical implications.

Anderson GG, Goller CC, Justice S, Hultgren SJ, and Seed PC. Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. Infect Immun 78: 963-975, 2010.

Bladder Control in Women: A recent study reported that two common operations for stress urinary incontinence (SUI) help women achieve similar levels of dryness. SUI is the leakage of small amounts of urine during physical activity, such as coughing, sneezing, and exercising. This condition is commonly treated with surgery that is designed to provide additional support to the bladder neck and urethra during increases in abdominal pressure that occur with these activities. The two most common surgical procedures are called the retropubic sling and the transobturator sling. In both procedures a synthetic mesh material is implanted to act as a hammock, or sling, to support the urethra and prevent leakage. The retropubic sling places the mesh material under the urethra and behind the pelvic bone, while the transobturator sling places the mesh material under the urethra and out through the upper inner thigh or groin area. Although both mid-urethral sling surgeries have been approved by the Food and Drug Administration and have been performed in the U.S. for more than a decade, the overall comparative effectiveness of these clinical procedures was untested.

The NIDDK's Urinary Incontinence Treatment Network conducted the Trial of Mid-Urethral Slings by randomizing 597 women with SUI to receive either retropubic or transobturator sling surgeries, and the outcomes of the surgeries were compared. Twelve months after surgery, women who received the transobturator sling and women who received the retropubic sling had equivalent levels of treatment success: 78 to 81 percent of women achieved dryness as defined by no leakage during a stress test and a 24-hour pad test, and they had no additional treatment for the problem. Participants also completed validated questionnaires and a 3-day voiding (bladder emptying) diary, and reported additional treatment with surgery, behavioral therapy, or drug therapy. Results from the questionnaire showed that 62 percent in the retropubic group and 56 percent in the transobturator group reported they had been cured.

Each type of surgery had different risks and side effects. Serious adverse events were more common in the retropubic group (14 percent), compared to the transobturator group (6 percent). More bladder perforations during surgery and serious voiding problems requiring surgical correction occurred in the retropubic group, while more vaginal perforations during surgery and neurological problems like weakness of the upper leg occurred in the transobturator group. Blood loss during surgery, duration of surgery, and likelihood of post-surgery urinary tract infections were all modestly higher in the retropubic group, compared to the transobturator group.

This rigorous, large-scale trial represents a major milestone in treatment for stress urinary incontinence, an underdiagnosed public health problem affecting millions of American women. Investments in this kind of research enable women and their doctors to weigh more accurately the benefits and risks of available treatment options.

Richter HE, Albo ME, Zyczynski HM, et al. Retropubic versus transobturator midurethral slings for stress incontinence. N Engl J Med 362: 2066-2076, 2010.

Identification of Inhibitors of Crystal Growth in Kidney Stone Disease: Kidney stones are among the most painful—and, unfortunately, common—of urologic disorders. Now, scientists have uncovered new insights into how the stones form and grow, information that may lead to better treatments for a condition that accounts for approximately 3 million visits to health care providers each year.

Kidney stones are crystals, a type of structure in which the atoms or molecules that comprise it are arranged in an orderly, repeating pattern in three dimensions. The most common type of kidney stone is made up of calcium in combination with either oxalate or phosphate. Kidney stones composed of L-cystine—an amino acid that dissolves poorly in urine—while less common, tend to be larger, to recur more frequently, and to be more likely to lead to chronic kidney disease. There currently is no ideal treatment for L-cystine stones; current therapy consists of increased fluid intake to dilute the urine, modulation of salt intake to change the pH of the urine and make stone formation less likely, or sulfur-containing drugs that have unpleasant side effects.

Researchers used a powerful imaging technique called atomic force microscopy to observe, in real time, growth of L-cystine crystals in solution and to measure the rate of this growth. They found that addition of either of two chemically-modified derivatives of L-cystine—either L-cystine dimethylester (L-CDME) or L-cystine methylester (L-CME)—dramatically reduced the growth rate of the L-cystine crystals, with L-CDME appearing particularly effective. Further analysis revealed that this was because binding of the modified L-cystine molecules disrupted the ordered arrangement of the crystal and made it more difficult for additional molecules to join the structure.

Although this study did not address the therapeutic use of L-CDME for the prevention and treatment of kidney stones, the researchers suggest that the concentration of the molecule shown to inhibit crystal growth under experimental conditions is low enough that it might be achievable in people. Future research may show whether L-CDME, or other compounds designed to disrupt crystal formation, would be a viable prevention or treatment for L-cystine stones.

Rimer JD, An Z, Zhu Z, et al. Crystal growth inhibitors for the prevention of L-cystine kidney stones through molecular design. Science 330: 337-341, 2010.

HEMATOLOGY RESEARCH

Improving Cord Blood Stem Cell Transplantation for Patients with Various Blood Diseases: A team of researchers developed the first successful laboratory culture system for increasing or expanding the numbers of cord blood stem cells in order to shorten the time necessary for complete engraftment for bone marrow transplantation. Umbilical cord blood is a source of blood-forming cells used in transplants. However, its utility is restricted due to the relatively small number of stem cells in a unit of cord blood. Because of this limitation, compared with a conventional bone marrow transplant, cord blood transplants take longer to fully repopulate all the different types of blood cells in the body. The longer timeframe for engraftment places the patient at increased risk of acquiring life-threatening infections, owing to the inadequate number of white blood cells. For this reason, cord blood is used more often in patients with a small body size, for example children, as they require fewer cells. Patients with larger bodies may have to be transplanted with two or more units of cord blood and still may contend with engraftment times longer than conventional bone marrow transplant.

The investigators took advantage of their knowledge of the "Notch" signaling pathway which stimulates expansion (cell division) of stem cells. A protein was engineered in the laboratory that activates the Notch pathway. The protein was used to stimulate expansion of cord blood stem cells in culture. The presence of the protein resulted in a greater than 100-fold increase in cultured cord blood stem cells compared with cells grown in the absence of the protein.

The researchers then conducted a pilot study of 10 patients with leukemia to begin to assess the safety of infusing cord blood stem cells that had been expanded in the laboratory with this Notch-mediated procedure and to perform an initial evaluation of the engraftment properties of the expanded stem cells. Each patient received two units of cord blood—one unit of non-expanded blood and one containing expanded blood cells that had been expanded with this procedure or two units of non-expanded blood. In this small group of patients, the investigators reported that they did not encounter safety issues. The median time for engraftment using the expanded cells was 16 days versus 26 days when non-expanded units of cord blood were used.

The study's intriguing results suggest that engraftments derived from expanded cells may proceed more rapidly than those derived using conventional (non-expanded) cord blood. These results need to be followed up by a larger study in order to develop statistically significant results.

Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, and Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat Med 16: 232-236, 2010.

Potential New Strategy To Improve Bone Marrow Transplant Success Rate: Scientists have recently reported a translational research finding that may improve outcomes for patients undergoing bone marrow transplantation. Currently, granulocyte

colony stimulating factor (G-CSF) is used to mobilize hematopoietic stem cells (HSCs) from the bone marrow into the bloodstream where it is collected for use in bone marrow transplantation. Unfortunately, in up to 10 percent of donors, this procedure does not mobilize sufficient numbers of HSCs from the bone marrow, precluding self (autologous) transplantation in those donors, or at the very least delaying the recovery time from the procedure. Thus, additional approaches are needed to increase the number of HSCs in the circulating peripheral blood.

Using genetic and pharmacologic approaches in mice, researchers have discovered that the activation of cell-surface "epidermal growth factor receptor" (EGFR)—a protein that spans the cell membrane and transduces signals from outside the cell to inside it—inhibits the ability of G-CSF to mobilize HSCs. EGFR and the factors that bind to it are well-known cell signaling molecules involved in diverse cellular functions, including cell proliferation, differentiation, motility, and survival, and in tissue development. Mice carrying a mutation in their *EGFR* gene that results in diminished receptor activity showed greater HSC mobilization in response to G-CSF stimulation. Similarly, a pharmacologic inhibitor of EGFR activity, called erlotinib, increased G-CSF's ability to mobilize HSCs by three- to seven-fold in normal mice, depending on the dose of the EGFR inhibitor. Importantly, in mice lacking the *G-CSF* gene, erlotinib had no effect.

This pre-clinical study reveals a previously unknown role of EGFR in HSC mobilization and provides a new approach for improving mobilization through EGFR inhibition. Additional research will be necessary to evaluate this approach to improving bone marrow transplantation prior to its use in people, but it points toward a new pharmacologic means of improving transplantation outcomes.

Ryan MA, Nattamai KJ, Xing E, et al. Pharmacological inhibition of EGFR signaling enhances G-CSF–induced hematopoietic stem cell mobilization. Nat Med 16: 1141–1146, 2010.

Mesenchymal Stem Cells Get Good Neighbor Award: A team of researchers has discovered that mesenchymal stem cells (MSCs) are an essential component of the HSC niche, the microenvironment in bone marrow where the cells are found. HSCs, a type of adult stem cell, hold great promise for future biomedical applications because of their ability to self-renew and develop into any kind of blood cell. However, HSCs are estimated to be a very rare cell type in bone marrow—only 1 in every 20,000 cells. Current scientific inquiry seeks to further our understanding of how the niche environment maintains HSCs with the hope of one day being able to manipulate the HSC population for potential use in various therapeutic interventions.

Scientists identified a novel cell type in bone marrow—the MSC—through its production of a protein called "nestin." They observed that MSCs outnumber HSCs 10 to 1 in bone marrow and are in direct contact with or cluster around HSCs, suggesting that MSCs might contribute to maintenance of HSCs. In fact, MSCs were found to make an abundance of supportive factors for HSCs. They were also found to self-renew, a key characteristic of stem cells.

To obtain more evidence about whether MSCs maintain or support HSCs in the bone marrow of mice, MSCs were depleted from their normal levels in mice using genetic and pharmacologic means. The result of fewer MSCs was an approximate four-fold reduction in HSC metabolic activity—evidence that MSCs play an important supportive role for HSCs. To evaluate the possible role of MSCs in homing HSCs to the marrow, HSCs were transplanted into mice whose MSCs had been depleted. Homing of HSCs to the marrow was reduced by 90 percent, and those cells that did home to the marrow tended to be located near MSCs, indicating that MSCs participate in the migration process.

This study illustrates the importance of the neighboring MSC to the HSC and provides additional information regarding a unique niche in the bone marrow made up, in part, by these two adult stem cell types. Future research efforts may explore the pharmacological targeting of the niche to enhance HSC production for use in regenerative therapies.

Méndez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466: 829-834, 2010.

STORY OF DISCOVERY

Sickle Cell Disease

Sickle cell disease is an inherited, chronic, and painful blood disorder. In the U.S., approximately 70,000 to 100,000 people have sickle cell disease,[1] predominantly individuals of African descent. Worldwide, millions of people have this disease. People with sickle cell disease have episodic severe pain in their bones, joints, and other parts of the body, as well as leg ulcers, jaundice, organ damage, and other serious health problems that may lead to multi-organ failure. The episodic, severe pain and complications associated with this disease can have a profound impact on the quality of life, ability to work, and life span of affected individuals. However, there is considerable variation in the clinical manifestations and severity of sickle cell disease among patients that is not completely understood. It is estimated that one of every three people with sickle cell disease who are hospitalized for pain will return to a hospital or emergency department within 30 days because of recurrent pain.[2] In the U.S., the average life expectancy for individuals with sickle cell disease has improved in recent decades but is still only 42 years for men and 48 years for women.[1]

What is Sickle Cell Disease?

Understanding of sickle cell disease at the molecular and cellular level began 100 years ago. In 1910, Dr. James Herrick, a Chicago physician, observed that the red blood cells of a patient from the West Indies were "sickle" shaped. It was later discovered that this red blood cell abnormality characteristic of sickle cell disease is caused by a mutation in hemoglobin, a protein that gives red blood cells their color and carries oxygen as these blood cells circulate throughout the body. Normal hemoglobin, referred to as hemoglobin A or HbA, consists of four protein components—two alpha globin chains and two beta globin chains. In sickle cell disease, the beta globin chains have a slightly altered structure caused by a heritable, genetic mutation of the beta globin gene. This alteration causes hemoglobin to polymerize and assemble into rod-like structures when red blood cells release their oxygen to tissues. Polymerized sickle hemoglobin elongation distorts the red blood cell membrane such that the red blood cells develop a "sickle" shape. Because "sickle" red blood cells are stiff and may clump together, they can block blood flow in small blood vessels, causing muscle, bone, and joint pain and eventual organ damage. Individuals with sickle cell disease usually have severe anemia (low red blood cell numbers) because sickle red blood cells have a much shorter lifespan in the circulation than normal red blood cells, and the bone marrow cannot produce new red blood cells fast enough to compensate for the rapid destruction of sickle cells.

Pinpointing the Molecular Basis of Sickle Cell Disease

In an elegant series of experiments in the 1940s and 1950s, specific chemical differences between normal HbA and sickle cell hemoglobin (referred to as HbS) were discovered. First, Dr. Linus Pauling demonstrated that HbA had a more positive charge than HbS and could be readily distinguished from HbS by a technique called electrophoresis. Then, Dr. Vernon Ingram found that one of the 146 amino acids in the beta globin protein component of HbA was altered in HbS; specifically, he found that the glutamic acid at position 6 in HbA was replaced by a valine in HbS. Subsequently in the mid-1970s, NIH-supported scientists showed that this amino acid substitution was caused by a specific mutation of the beta globin gene in sickle cell disease.

This sickle cell disease mutation is inherited. Individuals with the disease have two copies of the "sickle" hemoglobin gene, inherited from each parent. However, when a child inherits a mutated gene from

one parent but a normal gene from the other parent, the child has what is referred to as "sickle cell trait" and typically lives an asymptomatic, normal life.

Past and Current Treatments of Sickle Cell Disease

Until the early 1990s, treatment approaches were limited primarily to pain management and prevention of infections. One variable found to influence the clinical severity of the disease was the relative amount of another form of hemoglobin, called fetal hemoglobin (HbF), in red blood cells. HbF consists of two alpha globin chains and two gamma globin (not adult beta globin) chains; this form of hemoglobin predominates during human fetal development. Because HbF contains gamma globin chains instead of beta chains, it is not affected by the genetic mutation that causes sickle cell disease. While levels of HbF decline to very low levels after birth, varying levels of HbF may persist in the red blood cells. In people with sickle cell disease, HbF, if present at sufficient levels, can reduce the tendency of HbS to form the rod-like structures within red blood cells and to cause "sickling."

In 1985, the drug hydroxyurea, which is used in cancer therapy, was shown to increase the levels of HbF in red blood cells. Subsequently, investigators in the Intramural Research Programs of the National Institutes of Health—including the current NIDDK Director, Dr. Griffin Rodgers—and at The Johns Hopkins University School of Medicine, conducted a clinical trial in adult patients with sickle cell disease to evaluate the ability of hydroxyurea to increase HbF in the red blood cells of these patients and to reduce the clinical manifestations of their disease. The results of their study, published in 1990, showed therapeutic benefit in 7 out of 10 patients and led to subsequent large multi-center studies that established hydroxyurea as an effective form of treatment for sickle cell disease. Although not all patients respond to hydroxyurea, those that do have an improved quality of life—they are often able to attend school or work normally and better enjoy the normal activities of daily life. While hydroxyurea was a major breakthrough in the treatment of sickle cell disease in adults—and remains the only Food and Drug Administration-approved treatment for this disease—it is not a cure and it must be taken continuously.

For children with sickle cell disease, transplantation of blood stem cells from a donor without this disease has been shown to be curative. However, this procedure is only performed when there is a bone marrow (or blood stem cell) donor whose immunologic tissue type matches that of the patient. Siblings without sickle cell disease can sometimes be immunologically matched and serve as transplant donors, as can, occasionally, unrelated individuals of the same racial background. However, most children with sickle cell disease do not have a sibling who is a potential donor for transplantation, and bone marrow registries do not currently have sufficient numbers of African American donors for matches to be identified for the many patients who might benefit from this procedure. Moreover, standard conditioning regimens used to prepare a patient for blood stem cell or bone marrow transplantation are very toxic, particularly for adult patients with sickle cell disease, and serious complications often occur following standard bone marrow transplantation.

New Hope for the Future

In 2009, a team of researchers reported results of blood stem cell transplantation in adult patients with sickle cell disease using a modified transplant regimen with greatly reduced toxicity that was developed to make this treatment approach safer and much less harmful to the patient. This study was conducted at the NIH Clinical Center by NIDDK researchers Dr. Rodgers and Dr. John Tisdale of the NIH Molecular and Clinical Hematology Branch, who led the study, as well as investigators from the National Heart, Lung, and Blood Institute and the National Institute of Allergy and Infectious Diseases. Instead of using standard high-dose chemotherapy to prepare patients to receive the donor blood stem cells, the researchers

used a relatively non-toxic, "non-myeloablative" preparative procedure that does not destroy the patient's own bone marrow. Rather, this new regimen, based on earlier research with mice, was designed to suppress immune responses of the patient only enough to allow donor blood stem cells to engraft. Moreover, after the blood stem cell transplants were delivered into the recipient, a modified immunosuppressive regimen was used to sustain the graft and to prevent post-transplant complications.

Around 2.5 years post-transplantation, the researchers reported in a December 2009 publication that all 10 of the adult participants who had sickle cell disease were alive and well, and 9 no longer suffered from clinical manifestations of sickle cell disease. The results of this transplantation study indicate that adult patients with sickle cell disease now have an additional—and potentially life-changing—treatment option if a matched blood stem cell donor is available.

This clinical trial represents a major milestone for developing a cure for sickle cell disease. According to Dr. Tisdale, if participants in this ongoing trial continue to do well, it may be possible to extend this treatment approach to the use of "haplo-transplantation" donors (that is, sibling or parent donors whose immunologic tissue type only half matches that of the patient). If this becomes possible, it would allow most people with sickle cell disease to be treated with a potentially curative blood stem cell transplant.

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[1] www.cdc.gov/ncbddd/sicklecell/about.html

[2] Brousseau DC, et al. Accute care utilization and rehospitalizations for sickle cell disease. JAMA 303: 1288-1294, 2010

Chronic Pelvic Pain: Opening the Black Box

Dr. Anthony Schaeffer

Dr. Anthony Schaeffer is the Herman L. Kretschmer Professor and Chairman of the Department of Urology at Northwestern University in Chicago, Illinois. Dr. Schaeffer has led pioneering work in basic and clinical studies of urinary tract infections and prostatitis, involving novel concepts regarding the cause and treatment of these conditions. Dr. Schaeffer has also made major contributions to the management of post-prostatectomy incontinence through the implementation of a mobile urethral sling procedure. Dr. Schaeffer earned his M.D. from the Feinberg School of Medicine at Northwestern University and interned at the Chicago Wesley Memorial Hospital, after which he pursued a surgery residency at McGaw Medical Center of Northwestern University and a urology residency at Stanford University Medical Center in California. Dr. Schaffer has been an NIH-supported researcher for the past 30 years, including research support from NIDDK for at least 20 of those years. At the September 2010 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Schaeffer presented on urologic chronic pelvic pain, sharing some new insights into what this condition might be.

Urologic chronic pelvic pain encompasses two major pain syndromes—interstitial cystitis/painful bladder syndrome, which primarily affects women, and chronic prostatitis/chronic pelvic pain syndrome, which only affects men. Both syndromes, however, share the characteristics of severe pain below the abdomen, often with urinary frequency and urgency, and in both cases their cause remains unknown. Research is revealing that millions of people worldwide may have symptoms of urologic chronic pelvic pain syndromes, with attendant suffering akin to patients with serious chronic illness. As fully effective treatments are elusive and there is no cure, people with these syndromes suffer and can also incur high medical costs for themselves and the health care system. Dr. Schaeffer described research suggesting that urologic chronic pelvic pain syndromes may be mediated by novel adaptations of well-known host-bacteria interactions, as well as evidence that the central nervous system can be permanently altered by these interactions—thus suggesting that these syndromes might actually be a disease of infectious origin.

Quest for a Possible Infectious Origin

Dr. Schaeffer related that, in many cases of urologic chronic pelvic pain, there appears to be an infectious beginning. In his experience, patients will recall contracting a urinary tract infection (UTI) prior to the onset of chronic pain symptoms. However, most of these people, when seen by a doctor later in life, have no detectable evidence of infection, and many of them do not have inflammation. So, how could a UTI, which is usually associated with acute, or short-term, pain, possibly trigger or transition into a chronic pain condition?

While one candidate might be the inflammation that results from infection, patient data suggest that there is a disassociation between infection, inflammation, and the presence of pain. For example, patients with urologic chronic pelvic pain syndrome may have pain but no current evidence of infection or inflammation. Also, whereas most patients with UTI experience pain that has long been assumed a natural consequence of infection-associated inflammation, some people have pain-free bacterial infections (*i.e.*, asymptomatic) who nonetheless also have inflammation. That is, there is evidence of inflammation, such as infection-fighting white blood cells in the urine, but no pain. So, Dr. Schaeffer and his colleagues performed experiments in animal models to determine if there

are differences between the bacteria that cause the acute, painful UTIs and those that are involved in asymptomatic infections. They did this by placing the different strains of bacteria—those from patients with either acute UTI or from patients with an asymptomatic infection—into mouse bladders, and then monitoring the pain the mice experienced over the course of an infection. They found that, indeed, only infection with the acute UTI strain caused pain. However, similar to observations in humans, other experiments showed that both strains were capable of causing inflammation—suggesting that the difference in their ability to incite pain lay elsewhere.

Searching for bacterial factors that could contribute to this difference, Dr. Schaeffer's team focused on lipopolysaccharide, or LPS. The LPS molecule, a large lipid-sugar molecule found on the surface of bacteria, is a so-called "virulence factor" that helps to optimize bacterial infection of a host. It is known to contribute to inflammation and shock, suggesting it might also somehow contribute to pain. Experiments with cells and in animal models revealed that LPS from either strain incited inflammation. When placed in mouse bladders, however, only LPS from the acute strain caused pain, and did so much more rapidly even than the acute infection itself. Dr. Schaeffer and his colleagues determined that the typical interaction between this molecule and a receptor on host cells, called Toll-like receptor 4, was indeed involved in mediating the pain response—a potential new function for this interaction, which typically mediates inflammation.

Molecular Studies and Potential Clinical Relevance

Through further analysis of the LPS molecule, Dr. Schaeffer and his team have uncovered some intriguing findings that suggest that a specific alteration in this molecule between different bacterial strains is somehow responsible for whether a bacteria induces an acute infection that can lead subsequently to chronic pain after the infection is cleared, or whether it only causes acute pain at the time of infection. Moreover, they now have evidence for how the LPS molecule may be used therapeutically. Shortly following infection with an acute UTI strain, mice were given either a mock treatment or LPS from asymptomatic bacteria. The LPS from the asymptomatic bacteria significantly reduced the pain associated with the UTI, implying a therapeutic response.

Dr. Schaeffer noted that they have found similar responses in mice in which they have caused interstitial cystitis-like symptoms using a herpesvirus. In other studies, bacteria isolated from a person with chronic prostatitis were transferred to the prostates of mice. These mice developed pain symptoms similar to human chronic prostatitis. Interestingly, the ability to cause pain symptoms also depended on the mouse model used in the experiments, suggesting that there are host-specific differences in susceptibility to pain. Moreover, Dr. Schaeffer and his team have observed in this spectrum of studies the same type of dissociation between inflammation and pain and infection as seen with the UTI and pain models.

Neuro-Mechanisms and the MAPP Network

In 2008, the NIDDK established the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network to enable multiple laboratories and investigators around the country to work collaboratively on novel ways of looking at these enigmatic syndromes. While the Network's focus is on two major forms of urologic chronic urologic pelvic pain syndromes, interstitial cystitis and chronic prostatitis, researchers are also exploring the possible relationship between these and other pain syndromes, including irritable bowel syndrome, fibromyalgia, and chronic fatigue syndrome. Through this Network, Dr. Schaeffer's group has begun to work with neurophysiologists and neuroimaging experts, using an imaging technology called functional magnetic resonance imaging (fMRI) to look at chronic pain states. For example, using a computerized system originally developed

by a back pain researcher to directly correlate a person's experience of pain with what is going on in the brain, their fMRI studies have shown some intriguing differences in brain activity between people experiencing acute pain from heat exposure, people with chronic back pain, and people with chronic prostatitis. These studies in people are made all the more intriguing by new studies in mouse models suggesting that, when instilled into the mouse bladder, UTI bacteria that cause chronic pain can also cause a persistent change in electrical signaling by part of the central nervous system.

In addition to apparent functional changes in the brain, Dr. Schaeffer and collaborators have examined changes in the brain structure of people with urologic chronic pelvic pain syndromes. These imaging studies reveal an apparent correlation between the intensity and duration of pain and the density in the brain's gray matter in different brain regions. Interestingly, some of the brain areas that appear to be affected by urologic chronic pelvic pain are important in human function and behavior, particularly in emotional decision making.

Summary and Future Directions

Dr. Schaeffer noted that the studies he presented provide evidence that there may be an infectious basis for the chronic pain experienced by people with urologic chronic pelvic pain syndromes, and that this pain persists well after the initial infection and inflammation has cleared. At a cellular level, host Toll-like receptors appear to be acting as novel "nociceptors" for pain in a way that is independent of inflammation. The bacteria appear able to modulate the pain response via differences in LPS, and there appears to be involvement of the central nervous system. Building on some of these new findings and other studies, Dr. Schaeffer's team is now exploring "designer bacteria" or bacterially based molecules that could be administered to alter the pain response in patients, providing hope that a better understanding of the genesis of pain in these conditions could lead to new treatments.

PATIENT PROFILE

Lindsey Duquette

For the Family of a Child with Nephrotic Syndrome It's Been a Real Roller Coaster Ride

Lindsey Duquette

August 27, 2004, was Pam and Jim Duquette's 10th wedding anniversary. "It also just so happened to be the date our roller coaster nightmare began," says Pam. It was on that day that the Duquette's up-to-then perfectly healthy two-and-a-half year-old daughter, Lindsey, was diagnosed with nephrotic syndrome, a condition in which damage to the kidneys causes large amounts of protein to leak from the blood into the urine. Nephrotic syndrome affects the tiny filtering units in the kidney, called glomeruli, and over time may lead to kidney failure and the need for dialysis or a kidney transplant. Currently, there is no known cure for nephrotic syndrome. Even removing the diseased kidneys and replacing them with transplanted organs does not guarantee that the disease will not return.

Lindsey is now a happy 9-year-old who loves gym, Silly Bandz®, and going to school—normal for most kids her age. But these are relatively new experiences for a little girl who for years has suffered the pain and trauma of nephrotic syndrome. In Baltimore, MD, Lindsey's mother spoke to approximately 50 researchers and others from the Nephrotic Syndrome Study Network (NEPTUNE), which seeks to find better diagnoses and treatments for nephrotic syndrome and to understand its primary causes. Pam related Lindsey's and her family's experience with the disease, while Lindsey distributed brochures and introduced herself individually to everyone present.

Nephrotic syndrome is sometimes the first sign of an underlying disease that damages the kidney's tiny blood filtering units, called glomeruli, where urine is made.

Lindsey's Story

It all started about 6 years ago, when Lindsey began waking up with puffy eyes. The Duquettes didn't think much of it. "As a child, I had allergies," says Pam, "so I assumed that that's what it was." Lindsey then started asking for ice, which is sometimes a sign that a person's body is low on iron, although Pam and Jim didn't know this at the time. Within days, Lindsey's feet began to get puffy. Pam contacted her pediatrician, but the doctor was not overly concerned about Lindsey's condition.

About 10 days later, the Duquettes were attending a New York Mets baseball game when they realized

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something might be seriously wrong with their daughter. Lindsey's face, arms, and legs were swollen, and she seemed to have difficulty walking. A friend's wife, who was sitting with the Duquettes during the game and happened to be a pediatrician, said to Pam, "I don't want to alarm you, but I think it might be nephrotic syndrome."

Nephrotic syndrome is sometimes the first sign of an underlying disease that damages the kidney's tiny blood filtering units, called glomeruli. The glomeruli filter waste products and excess water and salts into the urine while keeping proteins and other larger molecules in the blood. Damaged glomeruli may allow protein to leak from the blood into the urine. In fact, elevated protein levels in the urine are one sign of kidney damage. The leakage of protein into the urine causes a corresponding drop in blood protein levels, which can lead to fluid retention in body tissues and swelling.

After calling Lindsey's pediatrician from the stadium, the Duquettes rushed Lindsey to the nearest emergency room, where she was diagnosed with nephrotic syndrome. At the time, Lindsey's blood protein levels were critically low, putting her at risk of going into shock. Lindsey was rushed from the hospital in Queens to a hospital in the Bronx, where she spent the next 5 days receiving high doses of steroids to help modulate the immune response that triggers some forms of nephrotic syndrome. Three weeks later, she was once again hospitalized, this time for peritonitis, a potentially life-threatening inflammation of the membrane that lines the wall of the abdomen, and a complication sometimes associated with nephrotic syndrome. Lindsey spent 3 days in the pediatric intensive care unit (PICU) battling peritonitis. After being released from the PICU, she remained hospitalized for more than 2 months. "We got nothing but bad news the entire time Lindsey was in the hospital," says Pam. "Blood clots….high blood pressure….sky-high triglycerides….poor breathing to the point that Lindsey had to be placed on oxygen. It was petrifying." Jim, who is now on the board of the NephCure Foundation, divided his time between work and taking care of the couple's two older children, while Pam spent most of her time at the hospital with Lindsey.

The Roller Coaster Ride Continues

After 76 days of treatment, Lindsey's condition finally went into remission. However, for more than 2 years, the roller coaster ride continued, with Lindsey's nephrotic syndrome flaring up, then going back into remission. During all this time, Lindsey continued to take steroids and other immunosuppressants, which had devastating side effects: Lindsey stopped growing, her eyebrows became bushy, and the hair on her head became discolored, dry, and brittle.

In August 2006, the family moved to Baltimore. But things weren't getting much better for Lindsey. The long-term steroid therapy was causing her bones to weaken. She was in tremendous pain and "burning from the inside out," recalls Pam. "Her cheeks were all puffed out and she couldn't move as a result of the pain. I had to pick her up to take her to the bathroom." Lindsey was taking over 20 pills each day, including steroids and pain medications. Her small frame—all 40 inches and 60 pounds of her—was carrying 18 pounds of extra weight, also caused by the steroids. "Whenever we tried to wean her off the steroids, she'd relapse," says Jim. "It seemed like we were chasing our tails. Our daughter had no quality of life."

Currently there is no known cure for nephrotic syndrome. Even removing the diseased kidneys and replacing them with transplanted organs does not guarantee that the disease will not return.

"Mom, I just want to be a normal kid."

Physicians in Baltimore strongly recommended that the Duquettes approve having their daughter's two ailing kidneys removed, which meant Lindsey would either require a kidney transplant or be on dialysis for the rest of her life. In October 2008, the Duquettes

scheduled the procedure. Lindsey's reaction: "Mom, I just want to be a normal kid." "I just broke down and sobbed," says Pam. "It broke my heart."

On further reflection, the Duquettes reconsidered the surgery, both because nephrotic syndrome can reoccur in people with transplanted kidneys and especially because Lindsey's kidneys were otherwise functioning normally, which had always made her case an anomaly. They instead sought a second opinion. In the process they were referred, and were accepted, into a research study being conducted by physicians in NIDDK's Intramural Research Program on the NIH campus in Bethesda, MD. In many respects, it was a risky decision.

The research study that Lindsey is participating in, which is led by Dr. Jeffrey B. Kopp in the Kidney Disease Branch of the Intramural Research Program at NIDDK, is studying the effectiveness of novel anti-inflammatory therapies for treating patients with nephrotic syndrome and related diseases that do not respond to traditional steroid treatment. "The protocol called for Lindsey to be infused with rituximab," says Jim. Rituximab belongs to a class of drugs called monoclonal antibodies, and, like steroids, has serious side effects, including potentially life-threatening reactions. In addition, the long-term effects of the drug are unknown. However, after years of watching their daughter suffer, the Duquettes were desperate.

Fortunately for the Duquettes, Lindsey responded well to the rituximab. Nonetheless, it's been a rough ride. As of this writing, Lindsey has had nine total infusions of the powerful drug, which is designed to destroy the B cells in her body. Every 4 months, as the B cells repopulate, Lindsey's disease relapses. In addition, in March 2009, she started experiencing severe headaches resulting from inflammation in the back of the eye due to being taken off of steroids too quickly.

Things Take a Turn for the Better

But as fast as things seemed to be going badly, they seemed to turn around. By June 2009, Lindsey went into remission again. As of this writing, Lindsey has been steroid-free for 10 months. Her bones have started to regenerate and she is no longer going to physical therapy. She's down from her previous 20-plus pills each day to only two. Because she's no longer on steroids she's starting to grow again. She's lost the bushy eyebrows, and her hair is getting back to normal. She's also lost the 18 pounds of extra weight she gained. Best of all, after having been bed-ridden for the better part of 2 years, Lindsey has gone to school for a full year—and won attendance awards. "She's got tons of friends, gets invited to birthday parties, she can do back bends. She's a normal little girl," Pam says with great emotion.

The Duquettes know that they may not be off the roller coaster just yet. Rituximab is a powerful drug, and there's no telling what its long-term effects might be. But they feel they made the right decision to enroll Lindsey in the study. "Lindsey would not be in the position she is in today if it weren't for NIH," says Jim. "We've been treated with nothing but professionalism and kindness and have met some extraordinary human beings," Pam adds.

As for Lindsey, "she keeps asking me when she can go back to NIH," says Pam. "When I ask her why, she says 'I love their food.'"

Gaining a Better Understanding of Nephrotic Syndrome Through Research

Through research studies, such as the one that Lindsey is participating in, NIDDK is hoping to develop new and improved methods for treating people with nephrotic syndrome, which may ultimately lead to a cure. In addition to the rituximab treatment study, NIDDK also supports research efforts to better understand nephrotic syndrome and other glomerular diseases. The NEPTUNE study is collecting long-term

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observational data of patients with rare forms of underlying kidney disease that lead to nephrotic syndrome and combining this information with biological samples. The consortium's investigators hope to improve our understanding of the fundamental biology of the causes of these diseases and the factors that contribute to their progression. Concerted and innovative investigational strategies that combine basic, clinical, and translational science are expected to improve the diagnosis and treatment of these forms of kidney disease.

The Intramural Research Program of NIDDK conducts basic, translational, and clinical biomedical research related to: diabetes mellitus, endocrine, bone, and metabolic diseases; digestive diseases, including liver diseases, obesity, and nutritional disorders; kidney diseases; and hematologic diseases. More information on research conducted at NIDDK can be found at: www.niddk.nih.gov

The NIDDK, the NIH Office of Rare Diseases Research, and the NephCure and Halpin Foundations collaborate to support research on nephrotic syndrome and other glomerular diseases through the Nephrotic Syndrome Study Network (NEPTUNE). NEPTUNE is a multi-site, multidisciplinary collaborative research and education network designed to foster innovative approaches to the understanding of four glomerular disease areas: minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), and nephrotic syndrome due to other or unspecified cause. More information can be found at: http://rarediseasesnetwork.epi.usf.edu/NEPTUNE/index.htm

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