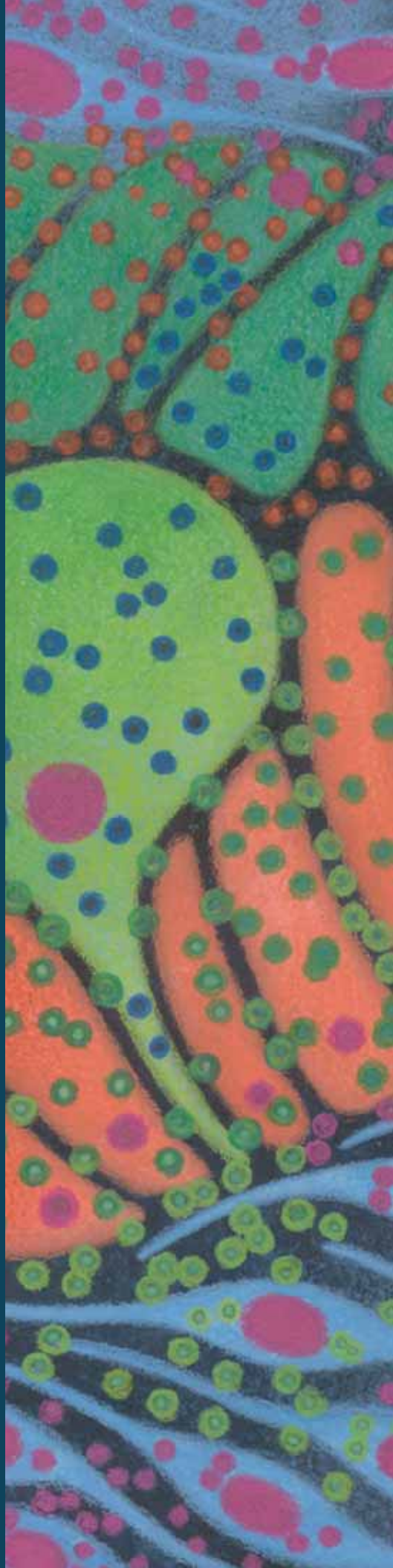
NIDDK

Recent Advances & Emerging Opportunities

February 2006



US Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

In May 2005, the NIDDK sponsored a workshop focusing on cellular niches—the factors and cellular microenvironments that modulate cell and tissue viability and function in health and disease. Cellular niches are critical regulators of stem cell differentiation; thus, knowing more about cellular niches is key to making progress in regenerative medicine. Participants discussed basic science and clinical issues, as well as new concepts for therapeutics. The workshop provided a forum for interactions among basic and clinical scientists, and included both established and new investigators in the field. The cover image is a detail of the poster that accompanied the meeting.

NIDDK

Recent Advances & Emerging Opportunities

February 2006

US Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

CONTENTS

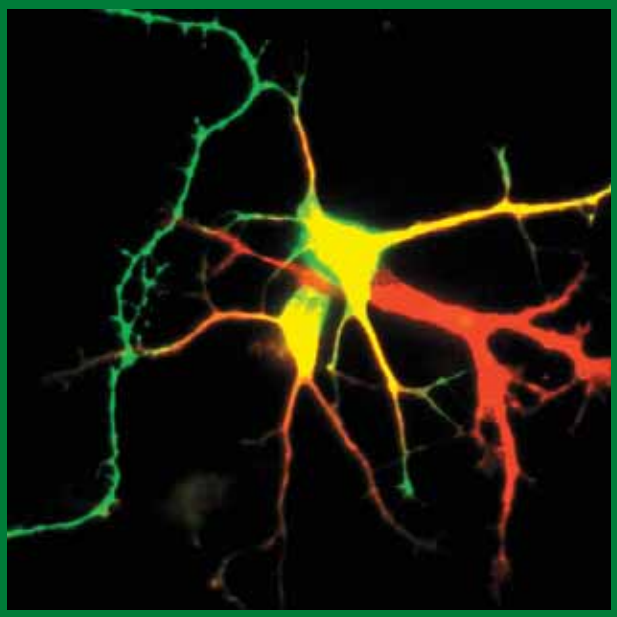
Message from the Director

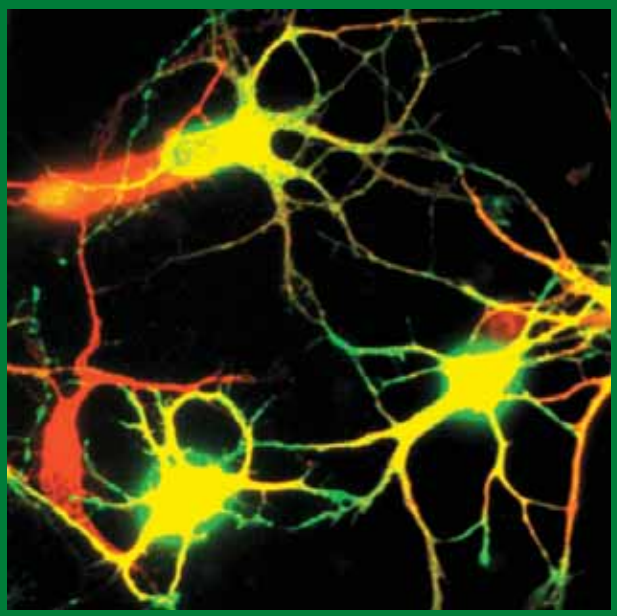
This annual publication features highlights of the research supported by the NIDDK on the diseases within its mission. The Institute's research scope encompasses many of the most costly and debilitating diseases and conditions affecting this Nation. These include diabetes, endocrine and metabolic disorders; obesity and nutritional disorders; and diseases of the digestive system, kidneys, urologic tract and blood.

By providing a "snapshot" of recent NIDDK-funded advances, this publication illustrates the benefits of our research investments to human health. For example, among this year's highlights, we report that intensive glucose control lowers the risk of heart disease and stroke by about 50 percent in people with type 1 diabetes, a result published in the December 22, 2005, issue of the *New England Journal of Medicine.* These findings emerged from a follow-up study of patients who took part in the Diabetes Control and Complications Trial more than a decade ago—thus demonstrating the continuing payoffs from long-term research investments. Another important recent development is that the U.S. incidence rates of irreversible kidney failure—also known as end-stage renal disease (ESRD)—have now stabilized after a 20-year climb. This hopeful finding points to the effectiveness of efforts to translate research discoveries into better means of diagnosis and early intervention. However, it is important to emphasize that these research benefits have not yet been realized across the entire U.S. population. Minority populations continue to bear a disproportionate burden from ESRD, and from type 2 diabetes, its frequent precursor. Thus, we are intensifying the activities of our National Kidney Disease Education Program and our National Diabetes Education Program to tailor and direct messages to minority populations regarding disease risks and science-based prevention and treatment strategies.

In addition to reporting on recent advances, this publication traces the multi-step path to research achievements through several "Stories of Discovery" and "Scientific Presentations." These essays illustrate the ways that incremental insights combine to form a continuum of progress. Complementing these scientific summaries are several personal stories of patients who suffer from diseases within the NIDDK's purview. These individual profiles tell of hope and determination, but also underscore the challenges that still must be overcome to conquer these diseases. Many of the diseases within the NIDDK mission are chronic. They cause years of affliction, with symptoms that range in severity, often with long-term detrimental effects on quality of life. Several, including type 1 and type 2 diabetes, chronic kidney disease, and hepatitis C, can ultimately lead to organ failure and death. Collectively, these diseases affect many millions of Americans, and cost hundreds of billions of dollars annually in direct medical costs and indirect costs resulting from disability, lost work and premature mortality. Given the overwhelming burden of chronic diseases, it is imperative that we develop effective strategies to preempt their onset and halt or slow their progression by propelling the rapid translation of new research discoveries into interventions that will directly benefit those at risk for disease, current patients, and future generations.

Allen M. Spiegel, MD
Director, National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Department of Health and Human Services





"MicroRNAs" are small inducible molecules that regulate a variety of biological processes. These images show the effect of higher-than-usual levels of one type of microRNA, *miR132*, on the development of cultured rat nerve cells. Nerve cells, which are stained yellow in both panels, send out projections, or neurites, to enable them to communicate with other nerve cells. Scientists engineered nerve cells to express elevated levels of *miR132*. These engineered nerve cells sprout more neurites (bottom) compared to cells with lower *miR132* levels (top). For more information about new insights into the role of small RNA molecules in cellular function, see the Scientific Presentation, "Role Reversal: RNA Control of Gene Expression," in this chapter.

Image courtesy of Dr. Richard Goodman. Photo credit: Soren Impey.

Cross-Cutting Science

Though advances sometimes happen in dramatic leaps and bounds, scientific progress more often occurs in incremental steps, with each new level of understanding building on previous discoveries. Insights into the nature of the fundamental and basic molecular components of an organism—its DNA, genes, proteins, and metabolites—and the exquisitely complex ways in which these elements are organized, regulated, and interact, provide a starting point for a wide range of inquiry. While ultimate application of this research may not always be immediately obvious, it forms the crucial foundation for future investigations, and knowledge gained from this research can be expected to facilitate disease-based research in a wide range of fields. A critically important aspect of cross-cutting research is its translation from research advances made in the laboratory into more effective therapies for patients. An equally important aspect of translational biology is the use of insights gained from clinical studies to spur novel research directions in the laboratory. The bi-directional flow of information, from "bench-to-bedside-and-back," allows scientists to address the widest range of research questions.

NIDDK TRANSLATIONAL RESEARCH: OPPORTUNITIES TO IDENTIFY AND VALIDATE BIOMARKERS

Biomarkers represent valuable surrogate endpoints for diseases and can be used in clinical trials and with diagnostic tools. Good biomarkers correlate well with disease state or progression, allowing physicians and researchers to readily gauge a patient's status at various disease stages and monitor the effectiveness of treatments. The NIDDK has a long track record of successfully promoting the development of biomarkers that have transformed patient care for a number of diseases within its research mission. For example, the hemoglobin A1c (HbA1c) blood test has been shown to be a good surrogate measure of long-term blood sugar control in diabetes. HbA1c has been validated in a large NIDDK-funded clinical trial, and subsequently has served as the basis for approval of multiple drugs for therapy of diabetes. Similarly, methods for estimating glomerular filtration rate (GFR) using circulating levels of the protein creatinine and earlier ascertainment of kidney disease by measuring the level of the protein albumin in the urine have become important surrogate markers for kidney function and disease.

However, additional biomarkers are urgently needed to speed development of potential new treatments.

Studies designed either to validate candidate biomarkers or to develop new technologies to monitor disease progression are particularly valuable and of special interest. For diseases for which no validated biomarkers are currently available, or for which measurement of well-characterized biomarkers is prohibitively invasive or expensive, the development of new biomarkers is particularly critical. To aid in this endeavor, the NIDDK has created a central repository with biological samples from individuals with a variety of the Institute's mission-specific diseases (*https://www.niddkrepository.org/niddk/home.do*). These samples are an extremely valuable resource made available to qualifying investigators who are pursuing research into biomarker discovery or validation.

Recent research has suggested a variety of specific biomarkers that may be valuable as surrogate endpoints for clinical trials of interventions for NIDDK mission-specific diseases. These may be particularly fertile areas for rapid translation, because they represent situations where a broad field of possibilities has been narrowed to a few promising candidates.

Examples of ongoing NIDDK-sponsored studies of potential biomarkers include:

C-peptide as a Biomarker of Beta Cell Function: In type 1 diabetes, residual beta cell function is associated with better glycemic control, less risk of hypoglycemia, and lower risk of long-term diabetes complications such as diabetic eye disease. In clinical trials designed to preserve beta cell function in individuals with new onset type 1 diabetes, it is critically important to be able to measure that function quantitatively. To achieve this, the NIDDK is working with the Centers for Disease Control and Prevention to improve sensitivity and standardization of assays for C-peptide—a byproduct of endogenous insulin secretion—and is conducting a clinical study to determine which of two methods is better to stimulate C-peptide for use as a surrogate outcome measure in clinical trials.

Antiproliferative Factor as a Biomarker for Interstitial Cystitis: Interstitial cystitis is a chronic inflammatory bladder disease of unknown etiology that primarily affects women. Symptoms include difficulty urinating, pain on urination, urinary urgency and increased frequency of urination. Antiproliferative factor is a protein found in the urine of patients with interstitial cystitis, and is therefore potentially valuable as a diagnostic biomarker, and as a surrogate endpoint for clinical trials.

Cystatin as a Biomarker for Kidney Function: Elevated levels of serum creatinine indicate lower glomerular filtration rate (GFR) and impaired kidney function. The development of an equation for estimating GFR from creatinine in the blood was a major advance for assessment of kidney health. While measurement of creatinine remains the best available means of estimating GFR, new research suggests that serum cystatin may provide a more accurate measure.

Other diseases and conditions for which the NIDDK is actively seeking to fund biomarker research include:

- Kidney and liver fibrosis
- Early diabetic microvascular complications
- Inflammation of the kidney
- Beta cell mass and inflammation of the pancreas
- Inflammation of adipose tissue
- Inflammatory bowel disease
- Hepatitis
- Insulin resistance
- Angiogenesis

All of the above are explicit goals of the "Development of Disease Biomarkers" research solicitation, released April 27, 2005, and active through September 1, 2008.

Other NIDDK initiatives relevant to biomarkers include:

Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases—This initiative takes a broad approach to finding new candidate biomarkers by examining protein expression in bio-fluids (e.g., plasma, serum, urine, bile, pancreatic and gastrointestinal fluids). One specific goal is to find a less burdensome test for diagnosis of diabetes that does not require fasting.

Non-Invasive Methods for Diagnosis and Progression of Diabetes, Kidney, Urological, Hematological and Digestive Diseases—In addition to non-invasive (e.g., imaging) methods, this initiative explicitly includes development of "minimally-invasive" methods, (e.g., through assays of bio-fluids).

Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) to Develop New Therapeutics and Monitoring Technologies for Type 1 Diabetes (T1D) and its Complications—This initiative seeks to promote the development of methodologies or biomarkers to help understand the physiology or improve the diagnosis and treatment of type 1 diabetes and its complications (e.g., applications of proteomic technologies, and development of better predictors of the patients with diabetes most prone to accelerated development and progression of macrovascular complications).

Research Grants for Studies of Hepatitis C in the Setting of Renal Disease—This initiative seeks to develop biomarkers or non-invasive means to assess the activity and stage of liver disease to avoid the need to perform liver biopsy in patients with kidney disease on dialysis or after kidney transplantation.

Calcium Oxalate Stone Disease—This initiative seeks to identify biomarkers that signal susceptibility to development of calcium oxalate stone disease or specific complications of this disease.

Mechanisms of Alcoholic and Nonalcoholic Fatty Liver (Steatosis)—This initiative seeks proposals

designed to develop non-invasive biomarkers for fatty liver, using genomic, proteomic, and metabolomic technologies.

Mechanisms of Alcoholic Pancreatitis—A specific goal of this initiative is the identification and characterization of biomarkers of early cell or tissue perturbation that can be used for diagnosis of alcoholic pancreatitis.

In addition to these ongoing initiatives, additional opportunities in biomarker research will also be addressed in several NIDDK initiatives in fiscal year 2006:

- Toward Imaging the Pancreatic Beta Cell in People
- Biomarkers of Autoimmunity in Type 1 Diabetes
- Collaborative Research in Proteomics of Obesity: A Search for Co-Morbidity Biomarkers

Current NIDDK-funded biomarker research includes, but is not limited to:

- Proteomic and metabolomic approaches to finding new diabetes diagnostics
- Proteomics and metabolomics in type 1 diabetes and its complications
- Initiatives to promote imaging of pancreatic inflammation and beta cell mass

NIH ROADMAP FOR MEDICAL RESEARCH IN THE 21ST CENTURY

Developed with input from meetings with more than 300 nationally recognized leaders in academia, industry, government, and the public, the NIH Roadmap provides a framework of the priorities NIH as a whole must address in order to optimize its entire research portfolio. It lays out a vision for a more efficient and productive system of medical research. It identifies particularly compelling opportunities in three main areas: New Pathways to Discovery, Research Teams of the Future, and Re-engineering the Clinical Research Enterprise.

The NIDDK has played a major leadership role in the Building Blocks, Biological Pathways, and Networks (BBPN) Roadmap Implementation Working Group, and the NIDDK has made significant contributions to implementation of BBPN initiatives on proteomics and metabolomics technology development. These efforts have included international scientific workshops to develop and promote information standards and resource sharing in these rapidly growing fields. Improved tools in proteomics—the study of all proteins within cells—and metabolomics—the study of all metabolites, such as salts, sugars, and fats—can directly benefit the study of diseases within the NIDDK mission. For example, metabolomics could lead to the identification and validation of surrogate markers that correlate with stage or rate of progression of diabetes and its complications.

The NIDDK has taken a leadership role in a Roadmap pilot program to make available, on a competitive basis, certain governmental contractual resources for the preclinical development of small molecules. This program was first developed for cancer research, and was expanded by the NIDDK to cover type 1 diabetes. Now, the NIH-RAID (Rapid Access to Intervention Development) Pilot has been expanded under NIDDK leadership to cover all diseases. It is intended to reduce some of the common barriers that impede the translation of laboratory discoveries, and clinical trials of new therapeutic entities. During the pilot phase, proposals are limited to small molecule development, but it is anticipated that eventually the program may be expanded to include other therapeutic agents. At present, the program is managed by NIDDK staff. While the program is designed to benefit researchers in any NIH-supported research area, it should prove particularly helpful to NIDDK-supported investigators, many of whom are actively involved in the development of new treatment approaches.

The NIDDK serves as the lead institute in administering the NIH Roadmap *Short Programs for Interdisciplinary Research Training* initiative, which was intended to provide training for investigators at all levels of their careers. The NIDDK also participates in the administration of the Training for a New Interdisciplinary Research Workforce initiative, through which institutional training grants have been developed.

The NIDDK Director has served as a member of the Roadmap Implementation Coordinating Committee, providing leadership to the structuring of Roadmap initiatives; their funding, progress review, and evaluation; and means of staff recognition. The NIDDK has also furthered the Roadmap goal of "Re-engineering the Clinical Research Enterprise" within the Institute by: (1) developing central

repositories for patient biosamples and data from NIDDK-funded clinical studies and trials, and (2) developing and implementing policies for, and funding of, ancillary studies to specific large clinical trials.

RESEARCH TRAINING

Diabetes-Based Science Education in Tribal Schools: Type 2 diabetes is a serious, growing problem in minority groups, including American Indians. The *Diabetes-Based Science Education in Tribal Schools (DETS)* program is developing a national, science-based diabetes prevention education curriculum for American Indian students in grades K-12. One goal of the program is to enhance awareness and understanding of diabetes among students, families, community members, and teachers in order to prevent the disease and to help affected tribal members better manage their diabetes. A second goal of the program is to increase the numbers of American Indians entering the health research professions. The program is sponsored by the NIDDK in close collaboration with American Indian tribal schools, and the Indian Health Service, the Centers for Disease Control and Prevention, and the Office of Science Education of the NIH.

Programs To Increase Diversity in Biomedical Research in NIDDK Mission Areas: Many diseases and disorders that disproportionately affect the health of minority populations in the U.S. are NIDDK research areas, including diabetes, obesity, nutrition-related disorders, hepatitis C, gallbladder diseases, *H. pylori* infection, sickle cell disease, kidney diseases, and certain complications from infection with HIV. The NIDDK supports research to encourage specific efforts in these areas of health disparity, including noteworthy efforts to enhance translation of new or improved therapy into clinical practice.

Because racial and ethnic minorities are under-represented among investigators carrying out the NIDDK mission, the Institute has a number of efforts to engage minority scientists and foster their career development. These efforts are aimed at ensuring an adequate cadre of future minority researchers by supporting current investigators and encouraging junior scientists and students to pursue research careers. The *Network of Minority Research Investigators*, composed of current and potential biomedical research investigators, is designed to help minority investigators achieve career success while working on issues concerning health-related racial and ethnic disparities. *Pre-doctoral Fellowship Awards for Minority Students* (F31) provide support for research training leading to a Ph.D., M.D./Ph.D., or other professional degree in the biomedical sciences, behavioral sciences, or health services research. These fellowships are designed to enhance the racial and ethnic diversity of the research labor force in the U.S. Under the *Minority Supplements to Institutional National Research Service Awards* (T32) program, the NIDDK awards an extra position, designated specifically for a selected under-represented minority trainee—either pre-doctoral or post-doctoral—to an existing T32 award. That position then remains a part of the award for as long as the named individual is a member of the training program. Undergraduate students are eligible for a number of NIDDK programs designed to encourage minorities to pursue a research career. Awards through the *Short-Term Educational Program for Under-represented Persons (STEP UP)* offer research-education opportunities for minority students in an effort to encourage them to pursue a research career in an area of science relevant to the research mission of the NIDDK. The ten-week *Summer Internship Program* provides an opportunity for students to participate in research under the direction of preceptors in NIDDK laboratories. This program advances the state of biomedical knowledge and introduces the students to state-of-the-art laboratory methods.

Role Reversal: RNA Control of Gene Expression

Dr. Richard H. Goodman

Dr. Richard H. Goodman is the Director of the Vollum Institute, Oregon Health and Science University, and professor of Cell and Developmental Biology and Biochemistry and Molecular Biology. The main research focus of Dr. Goodman and his colleagues is to determine how extracellular and intracellular signals are integrated, and how they control the onset and level of gene expression. Dr. Goodman described studies from his laboratory and related developments by other scientists at the May 2005 meeting of NIDDK's Advisory Council. The following scientific highlights are based on Dr. Goodman's presentation.

The human genome is described as our book of life—a book whose "stories" consist of genetic messages that are encoded within DNA molecules and spelled by means of four chemicals represented by the letters A, T, G, and C. Strings of these letters specify the thousands of different kinds of proteins that perform essential tasks in cells, tissues, and organs. But only 1.5 percent of the DNA in the human genome codes for such proteins. Some of the non-protein-coding DNA helps to regulate whether or not a particular gene is turned on ("expressed"), and to what extent. As scientific exploration of the genome continues, sequences within the rest of the non-coding 98.5 percent of the genome—once referred to as "junk DNA"—are also being found to regulate gene expression, in ways only recently discovered. Dr. Goodman has contributed significantly to current knowledge of the regulation of gene expression.

The processes that control gene expression are critical to enabling cells to perform their functions—for example, insulin secretion by cells in the pancreas, or activities of cells in the central nervous system. Moreover, they permit the formation of these cells in the first place—along with the myriad of other different types of cells in the body. Increased understanding of the regulation of gene expression, integral to life processes, may spur ideas for new therapeutics for many health conditions.

Basics of Controlling Gene Expression

The overall process of selectively turning some genes on and others off across hundreds of different types of cells involves a variety of intricate molecular mechanisms. Some gene control mechanisms can be likened to switches: when a gene is switched "on," cells make the specific protein that it encodes. But when that gene is "off," its particular protein is not made. In each cell throughout the body, the amounts and types of proteins being made are carefully controlled. This control is necessary because the aggregate levels and activities of proteins determine how specific cell types function in different parts of the body—for example, the pancreas, brain, heart, or muscle. This tight control is critical at all times, including during embryonic development, when from a fertilized egg, hundreds of different types of cells take shape, forming specialized tissues and organs. Each different cell type turns on only particular genes while suppressing others, so as to make a characteristic suite of proteins.

Early in their research, Dr. Goodman and his colleagues focused their attention on the gene encoding the protein called somatostatin, by studying when and how this gene is turned on, or expressed. They reasoned that, by investigating in depth how this particular gene is controlled, they would elucidate mechanisms that control the expression of

various genes. Many researchers had previously found that, as an early step in expressing the somatostatin gene or any other protein-encoding gene, certain information must be transcribed from DNA—the master genetic blueprint—to create a similar but distinctive molecule called messenger RNA or mRNA. In this copying process, proteins called transcription factors first "read" signals generally located upstream from the protein-coding portion of the gene. This sequence information in the protein-coding portion is incorporated into RNA molecules, which then move to another part of the cell, where the encoded message they carry is "read" to make a specific protein—in this case, somatostatin. Essentially, the first step in "turning on" or "expressing" a gene is the cell's recruitment to the master DNA of the necessary transcription factors needed to make RNA copies essential for the translation of key genetic information into proteins.

While studying the somatostatin gene, Dr. Goodman and his colleagues identified a discrete and important regulatory segment, located next to the protein-coding sequence of this gene. This site turned out to be the place where a subsequently-identified factor called CREB binds, to help turn on the gene under certain conditions. (CREB itself is a protein.) They later found another factor, termed CREB-binding protein (CBP), a "co-activator" that assists CREB in turning on genes. These findings have led to new insights into the control of numerous genes.

Because CREB is involved in controlling expression of many genes in addition to somatostatin, Dr. Goodman and his collaborators very recently developed an innovative molecular search strategy to identify the entire set of sites within a mammalian genome where the CREB protein binds. This search strategy, they reasoned, would lead them to all the various genes that CREB regulates. Using this search strategy in a rat cell model, the scientists identified a very large number of CREB binding sites throughout the genome. Many of these sites were near genes not previously known to be CREB-regulated. Strikingly, some of these turned out to be genes that encode types of RNA molecules, called "microRNAs," which have critically important regulatory functions. MicroRNAs are very short RNA molecules that don't get read to make proteins, like messenger RNAs do. Instead, microRNAs have a regulatory function: they bind to messenger RNA molecules and either block protein synthesis directly or cause those mRNA molecules to disintegrate.

Thus, CREB not only influences expression of many protein-coding genes, but, based on the surprising results of Dr. Goodman's genome-wide search, CREB also appears to be involved in the expression of genes for a number of microRNAs. This is also notable because so little had been known about how cells turn on genes that encode microRNAs.

MicroRNA Molecules Offer Another Means for Regulating Genes in Cells

Dr. Goodman recounted that the first microRNA had been discovered by other researchers working on worms as a model system in the early 1990s. MicroRNAs have since been found in many other organisms, including mammals.

MicroRNAs regulate a variety of cellular functions, including processes during development to help early-stage cells turn into nerve or other types of cells. One surprising and potentially important finding for researchers working on diabetes is that a particular microRNA molecule, designated *miR375*, appears to regulate the secretion of insulin, a hormone produced in the pancreas. Insulin is required for regulating glucose levels in the blood. When people eat a meal, the pancreas releases insulin to redirect the surge of sugar in the blood, thereby maintaining appropriate blood glucose levels and redistributing this chemical fuel to other tissues and organs in the body where it is needed. However, disrupting insulin functions can lead to diabetes. Type 1 diabetes results when specialized insulin-producing cells in the pancreas are mistakenly attacked and destroyed by the

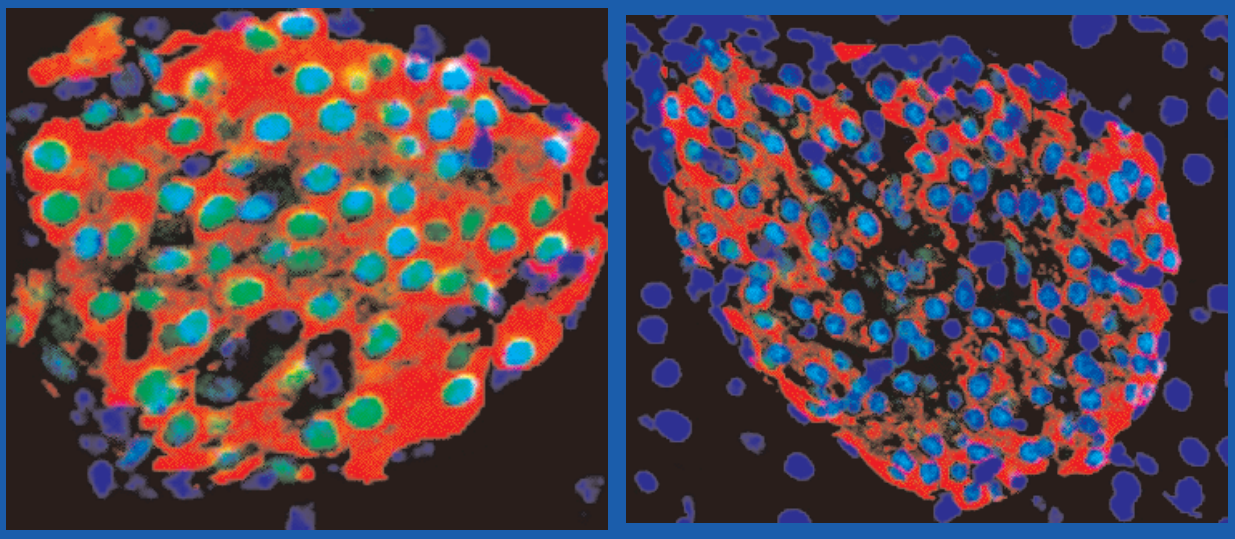
body's immune system. Even when insulin is being properly produced, individuals may develop type 2 diabetes when the body no longer responds appropriately to this hormone.

Dr. Goodman highlighted research by Dr. Markus Stoffel of Rockefeller University and his colleagues, who found that the *miR375* microRNA appears to inhibit glucose-stimulated insulin secretion in mouse pancreatic cells. It does this by interacting with the messenger RNA from another gene, which the scientists identified. The scientists also showed that experimentally inhibiting *miR375* molecules enhances insulin secretion. Thus, the discovery of the *miR375* molecule and its function may lead to a novel therapeutic approach for treating diabetes.

Dr. Goodman also pointed to exciting but preliminary research results from Dr. Gail Mandel's lab at SUNY Stony Brook showing that other microRNA molecules, along with CREB and another regulatory protein called REST, are involved in determining whether or not some cells become neural cells (neurons)—that is, part of the nervous system. They do this by turning some genes on, and others off. Dr. Goodman and his collaborators found that CREB helps turn on a microRNA that is involved in the development and specialization of neurons.

In contrast to CREB, which helps turn genes on, REST keeps genes turned off, including, for example, yet another microRNA gene, called *miR124*, that is needed in neurons. REST is present in both non-neural cells and in those precursor cells that are destined to become neural. In the latter cells, such as those in the brain, REST disappears during development but CREB persists; as a result, *miR124* and other factors can work to permit cells to express the genes needed to be neurons. However, in other cells—for instance, liver cells—REST persists and thus helps to prevent expression of neural-type genes. The *miR124* microRNA can shift cells towards becoming neural-like, apparently by destroying transcripts, or mRNA molecules, that ordinarily confer a non-neural character on those cells. If these findings are further supported by additional analysis, they will help to explain how highly specialized cells, tissues, and organs such as the brain and liver can arise from precursor cells and then maintain their new specialized identities. These findings also could prove instrumental as scientists study stem cells and learn how to modulate their functions to repair damaged tissues and organs.

By further elucidating how cells regulate gene expression and protein synthesis, Dr. Goodman's research is shedding light on critical biological processes relevant to development and health.



People with type 2 diabetes are less responsive than healthy people are to insulin—the hormone secreted when blood sugar rises. Many people with type 2 diabetes also produce reduced amounts of insulin, compounding the problem of insufficient insulin signaling. Recent research has indicated that an important underlying characteristic of those with the disease is that they have a reduced level of a protein called ARNT in their islets, the structures in the pancreas that produce insulin. These two images show histologic staining of a normal mouse islet (left) and an islet from a mutant mouse that lacks the *ARNT* gene (right). The genetic material (DNA) of the cells in the islets is marked by a blue fluorescent dye. The normal islet shows both the ARNT protein (marked with green fluorescent dye) and adequate production of insulin (marked with red fluorescent dye). In contrast, the mutant mouse islet that lacks the ARNT protein (green staining is absent) produces less insulin (red staining).

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, they affect many millions of Americans and profoundly decrease their 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 20.8 million people in the U.S.—over 7 percent of the total population—and is the sixth leading cause of death. Diabetes lowers average life expectancy by up to 15 years, increases cardiovascular disease risk two-to-four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult onset blindness. In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2002—including costs of medical care, disability, and premature death—was $132 billion. Effective therapy can prevent or delay these complications, but approximately one third of Americans with diabetes are undiagnosed.

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone which 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 to 10 percent of individuals with diagnosed diabetes. It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease, in which the immune system mistakenly attacks and destroys the beta cells of the pancreas. These beta cells, which are found within tiny cell clusters called islets, are the body's producers of insulin. If left untreated, type 1 diabetes results in death from starvation despite high levels of glucose in the bloodstream. 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 as well as they could if they had functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working on new beta cell replacement therapies meant to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for up to 95 percent of diabetes cases in the U.S. Type 2 diabetes is associated with several factors, including older age and a family history of diabetes. It is also strongly associated with obesity: more than 80 percent of people with type 2 diabetes are overweight or obese. Type 2 diabetes occurs more frequently among minority groups, including African Americans, Hispanic Americans, American Indians, and Native Hawaiians.

In patients with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. Gradually, the pancreatic beta cells secrete less and less insulin, and the timing of insulin secretion becomes abnormal. To control glucose levels, treatment approaches include diet, exercise, and medications; some patients also need to take insulin. There are also millions of individuals who have a condition called "pre-diabetes," in which blood sugar

levels are higher than normal, but not as high as in diabetes. This population is at high risk of developing diabetes. Fortunately, the Diabetes Prevention Program (DPP) clinical trial has shown that patients with pre-diabetes can dramatically reduce their risk of developing full-blown diabetes with improvements in lifestyle or with drug treatment.

Type 2 diabetes was previously called "adult-onset" diabetes because it was 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. 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, health care providers may find it increasingly difficult to strictly control a patient's blood sugar and thus prevent or delay the development of complications. Therefore, the advent of type 2 diabetes in youth has the potential to drastically worsen the enormous health burden that diabetes already places on the U.S.

The NIDDK is supporting research to better understand 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.

UNDERSTANDING TYPE 1 DIABETES

Insulin as an Autoantigen in Type 1 Diabetes: Recent exciting reports have suggested that the hormone insulin may be the critical initiator of the autoimmune destruction of insulin-producing pancreatic beta cells that leads to type 1 diabetes. Patients with the disease are known to have antibodies directed against insulin, and these antibodies are used to identify individuals at risk for the disease. However, it has been unclear whether insulin itself is the "key" autoantigen that triggers the autoimmune attack. Two new lines of evidence have now emerged—one in a genetically engineered mouse model of diabetes and the other using isolated T cells (a type of cell in the immune system) from pancreatic lymph nodes of people with and without type 1 diabetes. The first study found that diabetes did not develop in a type 1 diabetes mouse model engineered to express an insulin molecule not recognized by the mouse's immune system. The second study found that T cells from the lymph nodes of people with type 1 diabetes had large numbers of cells that recognized insulin, but those from non-diabetic persons did not. This research suggests that the immune systems of patients who are susceptible to developing type 1 diabetes do not respond appropriately to insulin—a hormone essential for life that all humans naturally produce. This derangement in recognizing insulin may provoke the immune system's misguided destruction of the body's own insulin-producing cells.

These studies are not the first to examine a possible role for insulin in the development of type 1 diabetes. The Diabetes Prevention Trial-Type 1 (DPT-1) studied whether injected or oral insulin administration could prevent or delay type 1 diabetes in persons at high- or moderate-risk for the disease. While the DPT-1 did not find an overall protective effect of injected or oral insulin, a subset of trial participants who had higher levels of insulin autoantibodies seemed to benefit from oral insulin treatment, though this result was not definitive. Plans are now underway for a trial to build upon the suggested benefit of oral insulin therapy in people with elevated insulin autoantibodies. This trial will be conducted by the Type 1 Diabetes TrialNet—an international network of investigators, clinical centers, and core support facilities that supports the development and implementation of clinical trials of agents aimed at slowing the progression of type 1 diabetes in new-onset patients and at preventing the disease in at-risk patients.

Kent SC, Chen Y, Bregoli L, Clemmings SM, Kenyon NS, Ricordi C, Hering BJ, and Hafler DA: Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. Nature 435: 224-228, 2005.

Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, Yu L, Wegmann DR, Hutton JC, Elliott JF, and Eisenbarth GS:

Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. Nature *435: 220-223, 2005.*

The Diabetes Prevention Trial-Type 1 Study Group: Effects of oral insulin in relatives of patients with type 1 diabetes. Diabetes Care *28: 1068-1076, 2005.*

Continued Benefits of Improved Blood Sugar Control: The landmark Diabetes Control and Complications Trial (DCCT) showed that intensive control of blood glucose levels reduced the risk of damage to small blood vessels and nerves in people with type 1 diabetes. The follow-up study, the Epidemiology of Diabetes Interventions and Complications (EDIC), continues to demonstrate long-term benefits of intensive therapy in these patients, and over 1,300 volunteers continue to participate. The EDIC study is now examining rates of cardiovascular disease among trial participants. During an average follow-up time of 17 years, the patients who had been intensively treated during the trial had fewer than half the number of cardiovascular disease events—heart attacks, strokes, or death due to cardiovascular disease—than those in the conventionally-treated group. These results show for the first time that intensive control of blood glucose levels has long-term beneficial effects on cardiovascular disease risk in type 1 diabetes patients. These findings are particularly significant because cardiovascular disease is the cause of death in two-thirds of patients with diabetes.

Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, and Zinman B for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group: Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med *353: 2643-2653, 2005.*

ADVANCES AND EMERGING OPPORTUNITIES IN TYPE 1 DIABETES RESEARCH: A STRATEGIC PLAN

In January 2005, the NIDDK convened an *ad hoc* planning and evaluation meeting of external scientific and lay experts in type 1 diabetes to perform a mid-course assessment of many currently funded type 1 diabetes research programs, and to identify future research opportunities within this context. One of the recommendations emanating from this meeting was to initiate a much broader review of the entire state-of-the-science with respect to type 1 diabetes, including recent research advances and emerging opportunities. In response to this recommendation, the NIDDK Director announced in March 2005 that the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC), chaired by the NIDDK, would spearhead a new type 1 diabetes strategic planning effort. The membership of this Committee includes all NIH components involved in diabetes research, as well as other relevant federal agencies.

The purpose of the Strategic Plan is to serve as a scientific guidepost to the NIH and to the investigative and lay communities by identifying compelling research opportunities that will inform the priority-setting process for the type 1 diabetes research field and propel research progress on the understanding, prevention, treatment, and cure of type 1 diabetes and its complications. The Strategic Plan is planned for release in Summer 2006.

INSIGHTS INTO TYPE 2 DIABETES

Gene Expression Changes Associated with Type 2 Diabetes: Although key aspects of the underlying disease process in type 2 diabetes are different from type 1 diabetes, both forms of the disease have in common a dysfunction of the insulin-producing beta cells of the pancreas. In advanced type 2 diabetes, it is not well understood how beta cells lose their ability to secrete insulin in response to high blood sugar (glucose) levels. To study the molecular mechanisms, scientists compared gene expression patterns in pancreatic islets—where beta cells reside—from people with type 2 diabetes and from those with normal blood glucose control. They observed a marked decrease in expression of a gene called *ARNT* (or *HIF1beta*) in patients with type 2 diabetes. *ARNT* encodes a transcription factor, a protein that can regulate the expression of many other genes. In cultured islet cells and a mouse model, diminished expression of *ARNT* or its absence in pancreatic islets resulted in defects in glucose-dependent insulin release and changes in gene expression patterns similar to those seen in patients with type 2 diabetes. *ARNT* controls many genes involved in diabetes, and these observations suggest a key integrating role for *ARNT/HIF1beta* in the

beta cell dysfunction that is associated with human type 2 diabetes.

Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ, and Kahn CR: Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic islet dysfunction in human type 2 diabetes. Cell 122: 337-349, 2005.

THE DIABETES PREVENTION PROGRAM—STILL YIELDING BENEFITS

DPP Continues To Underscore the Benefits of Preventing Type 2 Diabetes: Additional analyses of data from a landmark clinical trial have revealed more detailed information about the impact of the interventions. Researchers are continuing to gain new insights from the Diabetes Prevention Program (DPP) clinical trial, which examined the effects of lifestyle and medical interventions on the development of type 2 diabetes in adults at risk for this disease. The DPP compared intensive lifestyle modification, treatment with the drug metformin, and standard medical advice. Published in 2002, the DPP results showed that participants who received the lifestyle intervention had a dramatically reduced risk—by 58 percent—of developing type 2 diabetes. Metformin reduced diabetes risk by 31 percent. New data analyses show that hypertension, a classic risk factor for cardiovascular disease, was present in about 30 percent of all participants at the beginning of the study, and increased in the patients who received either placebo or metformin. However, hypertension significantly decreased in the lifestyle intervention group. Levels of recently identified, non-traditional risk factors for cardiovascular disease—such as C-reactive protein and fibrinogen—were lower in the metformin and lifestyle groups, with a larger reduction seen in the lifestyle group. About half of all DPP participants had a condition known as the "metabolic syndrome," which is defined by the presence of several conditions that increase risk for the development of type 2 diabetes and cardiovascular disease. Both lifestyle modification and metformin therapy reduced the development of the metabolic syndrome, with lifestyle modification more effective. Years later, the DPP continues to yield important insights into the prevention of type 2 diabetes in at-risk people.

Haffner S, Temprosa M, Crandall J, Fowler S, Goldberg R, Horton E, Marcovina S, Mather K, Orchard T, Ratner R, and Barrett-Connor E: Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. Diabetes 54: 1566-1572, 2005.

Orchard TJ, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S, and Fowler S: The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. Ann Intern Med 142: 611-619, 2005.

Ratner R, Goldberg R, Haffner S, Marcovina S, Orchard T, Fowler S, and Temprosa M: Impact of intensive lifestyle and metformin therapy on cardiovascular disease risk factors in the diabetes prevention program. Diabetes Care 28: 888-894, 2005.

PROTEOMIC AND METABOLOMIC APPROACHES TO THE DIAGNOSIS OF DIABETES

Biomarkers: The NIDDK supports an ongoing initiative to identify new biomarkers related to type 2 diabetes and pre-diabetes. Early diagnosis is crucial for reducing the overall burden of type 2 diabetes in the U.S. Many proteins and metabolites such as peptides, lipids and sugars may be modified in individuals with elevated glucose, a feature of pre-diabetes and diabetes. Disease-related changes in the protein profile of a cell (the proteome) and the metabolite profile (the metabolome) are ideal targets for new diagnostic techniques. Several novel proteomic and metabolomic technologies will be used to analyze plasma samples of pre-diabetic and diabetic patients. A simplified and less burdensome approach to the diagnosis of diabetes and pre-diabetes would facilitate increased recognition and improved care of these conditions.

METABOLIC PATHWAYS PLAY A KEY ROLE IN HEALTH AND DISEASE

Link Found Between a Saturated Fat Diet and Unhealthy Blood Fat: Researchers are uncovering the metabolic pathways that link several health problems. For example, elevated levels of fat and cholesterol in the blood are significant known risk factors for cardiovascular disease (CVD). Similarly, diets high in saturated and *trans* fats are strongly associated with high levels of fats and

LDL cholesterol ("bad" cholesterol) in the blood. In studying these types of associated health problems, scientists recently discovered a link between a diet high in saturated fat, changes in gene expression in the liver, and levels of fat and cholesterol in blood. In mice fed large amounts of saturated fat, they noted increased expression of a set of genes involved in fat synthesis in the liver, including one that augments the expression of many genes involved in fat metabolism, *PGC-1beta*. The protein encoded by this gene works with members of another family of genes (SREBP transcription factors) to help regulate fat synthesis in the liver. The *PGC-1beta* gene also influences the activity of a nuclear hormone receptor in the liver (LXR), which is involved in lipid and cholesterol metabolism in rats. A treatment to boost the *PGC-1beta*- regulated protein in the liver was found to reduce fat levels in liver, but to raise them in the blood, thereby suggesting that the protein activates pathways leading to fat export. The *PGC-1beta* gene therefore seems to lie at the nexus of two important pathways of fat metabolism in the liver: fat synthesis and export. These studies suggest that the *PGC-1beta* gene may be a good target for novel therapies aimed at reducing elevated circulating fat levels that arise from diets high in saturated fats.

Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, Yang W, Pei L, Uldry M, Tontonoz P, Newgard CB, and Spiegelman BM: Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. Cell 120: 261-273, 2005.

Newly-discovered Links Between Inflammation and Mediators of Metabolism: In recent years, researchers have uncovered intriguing links between the molecular mediators of inflammation and a number of human diseases. The enzyme IKK-beta is a central coordinator of inflammatory responses through its activation of the transcription factor NF-kappaB. Researchers studying severe muscle wasting that is often seen in AIDS, diabetes, and end-stage heart and kidney diseases, engineered mice that had a constitutively active form of IKK-beta in their skeletal muscle. These mice developed severe wasting, and inhibition of NF-kappaB activity could partially reverse this condition. Mice with the "always on" form of IKK-beta in their livers developed metabolic patterns similar to type 2 diabetes, with high blood sugar, severe insulin resistance in their livers, and moderate insulin resistance in muscle; drugs that block IKK-beta and NF-kappaB activity were able to ameliorate these conditions. In another study, mice were engineered to lack IKK-beta in either their livers or in myeloid cells, a type of white blood cell. In response to a high fat diet or obesity, mice lacking IKK-beta in their livers retained insulin sensitivity in this organ, but developed insulin resistance in muscle and fat. In contrast, mice lacking IKK-beta in their myeloid cells retained global insulin sensitivity under the same conditions. These observations suggest that IKK-beta acts locally in liver cells but systemically through myeloid cells to influence insulin sensitivity. Taken together, these results identify IKK-beta as a key player in modulating metabolism and insulin sensitivity. These studies also provide evidence that IKK-beta-mediated inflammation links obesity to insulin resistance. Drugs that target the inflammatory signaling pathway may be useful in treating both muscle wasting and insulin resistance.

Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, and Karin M: IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 11: 191-198, 2005.

Cai D, Frantz JD, Tawa NE, Jr., Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ, and Shoelson SE: IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119: 285-298, 2004.

Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, and Shoelson SE: Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 11: 183-190, 2005.

Umbilical-Cord Blood Cell Transplantation Slows Krabbe Disease Progression: Researchers have tested the use of umbilical-cord blood cell transplantation to treat Krabbe disease. This disease is a rare, inherited degenerative disorder of the central and peripheral nervous systems that is uniformly fatal, with many children dying before the age of two. It results from a deficiency in the enzyme that breaks down the molecule galactocerebroside, which is an important component of nerve tissue, leading to its accumulation and subsequent nerve damage. There is no cure for the disease, but replacing the missing enzyme in the brain has been predicted to be therapeutic.

Researchers have recently used umbilical-cord blood transplantation from closely-matched, but not identical,

donors to combat the disease. The hope was that transplanted cells in the cord blood would migrate to the brain and provide the missing enzyme and thereby halt nerve damage. The researchers treated two groups of newborns; one group was diagnosed with Krabbe disease before or at birth on the basis of family history and was transplanted in the first 6 weeks of life, and the other was diagnosed at the onset of clinical symptoms of the disease after birth and was transplanted in the first 6 months to 1 year of life. After 3 years of follow-up, when most untreated Krabbe patients would have died, survival was 100 percent for the newborns transplanted in the first 6 weeks of life, and 43 percent for infants transplanted after the onset of symptoms. Infants who underwent transplantation before the onset of symptoms did not develop the neurological impairment characteristic of the disease. Those who did not undergo transplantation until after the appearance of symptoms did not show neurological improvement. This research demonstrates the greatest benefit results from early intervention before clinical manifestations of Krabbe disease. Newborn screening should be considered for Krabbe disease to identify infants who would benefit from early transplantation.

Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S, Wenger DA, Pietryga D, Wall D, Champagne M, Morse R, Krivit W, and Kurtzberg J: Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. N Engl J Med 352: 2069-2081, 2005.

NIDDK AIDS RESEARCH

Research supported by the NIDDK has made important contributions to the current understanding of many of the metabolic complications associated with HIV infection and highly active anti-retroviral therapy (HAART). These complications include HIV- and HAART-related lipid dysregulation, insulin resistance, and abnormal body fat distribution. These complications are risk factors for serious diseases, such as diabetes and cardiovascular disease. The NIDDK also supports research to define the causes of liver disease associated with HIV. Areas of interest include the delineation of interactions between HIV and hepatitis B and C viruses, and the development of means to prevent and treat liver disease in HIV-infected persons. In addition, the NIDDK supports studies of the neurological, gastrointestinal, endocrine, renal, liver, and hematologic manifestations and complications of HIV infection. The NIDDK also maintains a highly productive intramural program on structural biology. Scientists seek to determine the structures of biologically significant proteins relative to HIV infection, replication, and integration.

Cardiovascular Disease Risk in HIV-Infected Women: New findings may help researchers overcome the metabolic complications sometimes associated with HIV infection and the highly active antiretroviral therapy used to treat the infection. It is not clear whether these complications arise from HIV infection per se, components of the antiretroviral therapy, or a combination of the two. These metabolic complications include lipid (fat) abnormalities, insulin resistance, and abnormal distribution of body fat. They are major risk factors for the development of serious diseases, such as diabetes and cardiovascular disease. These metabolic abnormalities have been previously well-studied in HIV-positive men, but relatively little information is available regarding their impact on women. A recent study shed light on cardiovascular risk factors in HIV-infected women relative to HIV-negative women. The infected women had higher levels of C-reactive protein (a marker of inflammation) and triglycerides (circulating fat levels); showed elevated 2-hour oral glucose levels after a glucose tolerance test; and had increased fasting insulin levels. Additionally, HIV-infected women had more abdominal visceral fat and less extremity fat than HIV-negative controls. Thus, the HIV-infected women had significantly increased risk factors for cardiovascular disease and abnormal fat distribution. These findings are expanding the knowledge base on which future studies may be framed for devising HIV treatment regimens that address metabolic complications of HIV infection and/or antiretroviral therapy.

Dolan SE, Hadigan C, Killilea KM, Sullivan MP, Hemphill L, Lees RS, Schoenfeld D, and Grinspoon S: Increased cardiovascular disease risk indices in HIV-infected women. J Acquir Immune Defic Syndr 39: 44-54, 2005.

Collaborative Islet Transplant Registry — Second Annual Report Published

Episodes of dangerously low blood glucose, or hypoglycemia, were greatly reduced in people who received islet transplants for poorly controlled type 1 diabetes, according to an analysis of outcomes in 138 patients who had the procedure at 19 medical centers in the United States and Canada. This is one of the conclusions of the Collaborative Islet Transplant Registry (CITR), which tracks many factors affecting the success of this experimental procedure in people with severe type 1 diabetes. The CITR released its second annual report (*http://www.citregistry.org*) in September 2005.

In the islet transplantation procedure, islets—which are clusters of cells that include the insulin producing beta cells—are extracted from a donor pancreas and infused into the portal vein of the recipient's liver. In a successful transplant, the islets become embedded in the liver and begin producing insulin.

The CITR reported that, one year after their last islet infusion, 58 percent of recipients no longer had to inject insulin. Those who still needed insulin a year after their last infusion had a 69 percent reduction in insulin requirements. One infusion of islets, though not always enough to keep blood glucose in the normal range, generally lowered insulin needs and alleviated episodes of severely low blood glucose in most patients.

Despite the progress seen in islet transplantation in recent years, it is still an experimental procedure currently reserved for adults who are unable to control their diabetes with conventional therapy. Islet recipients must take immunosuppressive drugs to prevent their immune systems from rejecting the transplant. Because of the adverse side effects of these drugs, the patients chosen for the procedure were those who had the greatest need and potential for benefit, such as those with a history of being unaware of hypoglycemia.

The scarcity of islets poses another major obstacle to wider testing of islet transplantation as a treatment for type 1 diabetes. Only about 3,500 pancreata are suitable for transplantation, and many of these organs are used for whole organ transplantation. To improve the potential of cell replacement therapy for diabetes, NIH-funded research is focusing on understanding the insulin-producing beta cell and its regeneration and on efforts to develop alternative sources of beta cells. Researchers are also working on ways to coax the immune system into accepting donor cells or tissues without suppressing the entire immune system.

The CITR's mission is to expedite progress and promote safety in islet transplantation by collecting, analyzing, and communicating data on islet transplantation. Single copies of the CITR report may be ordered free of charge from the registry (*http://www.citregistry.org*) or from NIDDK's National Diabetes Information Clearinghouse by calling 1-800-860-8747, or through the website at *http://catalog.niddk.nih.gov/detail.cfm?ID=824&CH=NDIC*

The National Diabetes Education Program (NDEP)

Through education and awareness campaigns and other health information dissemination efforts, the National Diabetes Education Program (NDEP) aims to improve treatment and outcomes for people with diabetes, to promote early diagnosis, and to help prevent the onset of diabetes. The program develops information and education messages and materials for people with diabetes and their families, health care providers, payers and purchasers of health care, health care system policy makers, and the general public—including people with undiagnosed diabetes and those at risk for the disease. The NDEP is jointly sponsored by the NIDDK and the Division of Diabetes Translation of the Centers for Disease Control and Prevention, both of the U.S. Department of Health and Human Services, and also involves the participation of over 200 public and private partner organizations.

In 2005, the NDEP continued to promote campaigns focusing on diabetes prevention and control. A key target audience for these messages is older adults. The NDEP launched its newly revised campaign for seniors to help them manage their diabetes. One campaign, "The Power to Control Diabetes Is in Your Hands," provides older adults with information on how to control their disease by regular self-monitoring of blood glucose and by following the "ABCs" of diabetes care to prevent or delay its serious complications. The "ABCs" refer to monitoring of hemoglobin A1c levels, blood pressure and cholesterol levels. The campaign also provides older adults with information on the newest Medicare benefits and how these benefits can help them manage their disease to live a long and healthy life. Educational materials include a consumer brochure, available in English and Spanish, and a community action kit, a comprehensive resource designed to assist community organizations in helping their older adult members living with diabetes.

Another NDEP campaign, "Small Steps. Big Rewards. Prevent Type 2 Diabetes," is based on the findings of the NIH-sponsored Diabetes Prevention Program (DPP) clinical trial. This trial demonstrated that the risk of developing type 2 diabetes can be significantly reduced through modest weight loss, of five to seven percent of body weight, along with exercise, such as 30 minutes of moderate physical activity five days per week. These lifestyle changes were most effective in adults over 60 years of age. In 2005, the NDEP reached out to older adults at risk for type 2 diabetes with the campaign, "It's Not Too Late To Prevent Diabetes. Take Your First Step Today," and developed tailored materials for seniors to motivate them to make modest lifestyle changes to prevent the disease.

In 2005, the NDEP added a new target audience to promote diabetes prevention messages—women with a history of gestational diabetes mellitus (GDM) and their children. An expert panel was convened to review the latest science on this topic, and a communication plan has been developed that targets health care providers and women with a history of GDM to educate them about how to lower their risk. The NDEP plans to launch this campaign in the spring of 2006 with educational materials for consumers and health professionals. For children at risk for developing type 2 diabetes, a new tip sheet, "Lower Your Risk for Type 2 Diabetes," was developed to alert at-risk children and adolescents and their families to take action to delay or prevent the onset of type 2 diabetes. To help teens with diabetes learn to cope with the emotional and psychosocial aspects of diabetes, the NDEP children's work group produced a tip sheet, "Dealing with the Ups and Downs of Type 2 Diabetes."

The NDEP has issued a new publication, "New Beginnings: A Guide for Living Well with Diabetes," to help groups facilitate dialogue about diabetes and its complications. This discussion guide, targeting African Americans with diabetes, has been developed to expand on the themes and educational opportunities brought out in "The Debilitator," a docudrama developed by an independent film company. "New Beginnings" was designed to be used by diabetes educators, church groups, clinics, hospitals, families, or anyone interested in talking about diabetes and its impact on African Americans.

In other activities, the NDEP continues to partner with the American Diabetes Association for the health awareness

campaign, “Be Smart About Your Heart: Control the ABCs of Diabetes,” in order to promote the link between diabetes and cardiovascular disease. In 2005, tailored materials were developed for American Indians and Alaska Natives, the newest target audience for the campaign. Versions of the campaign are also tailored for Hispanic and Latino Americans and for Asian Americans and Pacific Islanders. In November 2005, the NDEP partnered with the American Podiatric Medical Association on World Diabetes Day to promote messages about people with diabetes taking care of their feet to prevent lower-limb amputations.

The NDEP website addressing systems change to improve diabetes care can be accessed at: *http://www.betterdiabetescare.nih.gov*. The website is completing an update with new resources and tools. The NDEP is collaborating with the Indiana University School of Medicine (IUSM) to provide a continuing education component (CE) to the website through IUSM’s reflective learning program. The NDEP website for the workplace continues to include updated information on diabetes management and prevention topics for businesses and has new educational materials available in Spanish. It can be accessed at: *http://www.diabetesatwork.org*.

With the help of its 200 partners, the NDEP continues to reach millions of people with its messages. In 2005, messages about NDEP made more than 177 million media impressions through broadcast and print outlets, and the NDEP website received more than 1.6 million visits. To date, over one million copies of NDEP’s educational materials have been distributed through the National Diabetes Information Clearinghouse.

The NDEP evaluation work group continues to collaborate with its partners to collect data and track progress toward the program’s goal and objectives. Both process and outcome data measures are being monitored to help with program planning of future activities, campaigns and materials. The NDEP also conducts a semiannual online, web-based partner activity survey that shows how partners are disseminating program messages and resources.

The NDEP also offers other patient education materials, and resources and tools designed for health care professionals. The NDEP has begun an initiative to determine the economic impetus for diabetes prevention and control. Further information on all NDEP campaigns, activities and materials can be found on the NDEP website, accessible at: *http://www.ndep.nih.gov*.

STORY OF DISCOVERY

Glucagon-like Peptides Yield New Diabetes Therapies

By deciphering how the body maintains normal blood sugar levels, researchers are finding clues to combat diabetes. In healthy individuals, the beta cells of the pancreas respond to elevated levels of sugar in the blood by releasing insulin, and the insulin, in turn, causes cells to absorb sugar. The pancreas reacts to low levels of blood sugar, on the other hand, by releasing glucagon from its alpha cells, triggering the liver to release part of its store of sugar into the blood and turning off insulin production. These are crucial steps in normal metabolism. In diabetes, this exquisite regulation is disturbed either by loss of cells that produce insulin (type 1 diabetes) or by inadequate amounts of insulin to compensate for diminished responsiveness of cells to the hormone—mostly in muscle and fat (type 2 diabetes).

This fairly simple paradigm is by no means the whole story, however. In the 1960s, researchers showed that sugar triggers more insulin to be released if the sugar is absorbed through the digestive system rather than injected directly into the blood. The underlying reasons were a mystery, but scientists speculated that the presence of sugar or other food in the gut might trigger the release of some hormone that increases the pancreatic insulin response. The putative insulin production-promoting hormone or hormones were called "incretins." A vital clue to the incretin mystery was uncovered in 1982 by Dr. Joel Habener and colleagues when they cloned the gene that encodes the glucagon protein. Inspection of the gene revealed that it also encodes two other proteins similar, but not identical, to glucagon. These were called "glucagon-like peptides," or GLPs. The NIDDK-supported researchers later demonstrated that a truncated form of one of these, GLP-1, was able to act as an incretin: pancreatic beta cells release more insulin in response to sugar in the presence of GLP-1 than in its absence. Thus "Glucagon-Like Peptide 1" actually has effects somewhat opposite to those of glucagon, in that it helps to lower blood sugar. As expected for an incretin, GLP-1 is produced by intestinal cells when stimulated by the presence of food. In addition to its effect on promoting the insulin response, GLP-1 also has the important effect of slowing stomach emptying, essentially helping a person "feel full." More recently, mounting evidence suggests that GLP-1 can stimulate the multiplication of insulin-producing beta cells, while simultaneously protecting them from so-called "programmed cell death."

Properties of GLP-1 suggested that it might be of potential therapeutic benefit for some people with type 2 diabetes. By boosting their natural insulin secretion in response to food, GLP-1 could reduce their need for injected insulin or other therapies. Unfortunately, scientists soon discovered a major potential barrier to this approach: GLP-1 lasts only a very short time in the blood stream before it is digested by an enzyme called dipeptidyl peptidase IV (DPP IV). A solution to this problem was found in the unlikeliest of places: the venom of a lizard native to the Sonora Desert. At about the same time that the glucagon-like peptides were being discovered by NIDDK grantees, NIDDK intramural scientists studying the so-called "Gila monster" discovered that proteins in the lizard's venom stimulate the release of digestive enzymes by cells of the pancreas. Because the reptile typically goes months between meals, these proteins may serve to "jump-start" its digestive system when it feeds. Among the proteins isolated from the venom was one, designated exendin-4, with considerable similarity to GLP-1. Scientists showed

that exendin-4 and GLP-1 are both capable of stimulating gastric secretions in guinea pigs, though exendin-4 is the more potent of the two. Indeed, two labs have independently demonstrated that both proteins work by stimulating the same cellular receptor. Furthermore, exendin-4 is not digested by DPP IV, and therefore can last much longer in the blood than GLP-1.

The discovery and characterization of the GLPs and of exendin-4 are the fruits of basic research, much of which was funded by NIDDK. Based upon this critical foundation, pharmaceutical companies have developed an important new treatment for patients with type 2 diabetes. A synthetic version of exendin-4 (the manufactured form is referred to as "exenatide," but is chemically identical to exendin-4) was recently tested for therapeutic benefit in industry-supported randomized controlled clinical trials. The studies enrolled patients with type 2 diabetes whose blood sugar was inadequately controlled. Patients then received either standard treatment or standard treatment plus a high or low dose of exenatide. At the conclusion of the studies, those patients who had received exenatide were found to have maintained significantly healthier levels of blood sugar, and those receiving the high dose of the new drug had done better than those with the low dose. Another exciting finding was that the patients who had received exenatide lost weight compared to the control group. A possible explanation for the latter finding is the effect that GLP-1/exendin-4/exenatide have on slowing stomach emptying and creating a sense of fullness. This result is particularly important because type 2 diabetes is associated with overweight and obesity. Again, those receiving the higher dose of exenatide achieved the better result, losing more weight. In April 2005, the Food and Drug Administration approved exenatide as a supplementary treatment for type 2 diabetes in patients whose blood sugar is not otherwise well-controlled.

Scientists are eager to explore potential benefits of exenatide in treating or preventing type 1 diabetes. This disease results when a person's immune system attacks and destroys his or her own insulin-producing pancreatic beta cells. The landmark NIDDK-sponsored Diabetes Control and Complications Trial (DCCT) had demonstrated that a significant percentage of people with type 1 diabetes retain the capacity to produce a small amount of their own insulin. This finding suggests that, in some patients at least, the process of autoimmune beta cell destruction may be modestly offset by beta cell regeneration. NIDDK intramural researchers Drs. David Harlan and Kristina Rother are currently testing whether exenatide can help capitalize on the presumed natural regenerative capability in patients who, although they have had type 1 diabetes for several years, still produce some insulin. Study volunteers are receiving exenatide either alone or in combination with an immunosuppressive drug designed to blunt the continuing autoimmune attack on their beta cells. Researchers in the newly formed Clinical Islet Transplantation Consortium plan to test the value of exenatide in enhancing the viability of transplanted islets, and the Type 1 Diabetes TrialNet is developing studies to assess the potential of exenatide to prevent or delay onset of type 1 diabetes in patients with autoimmunity directed at the beta cell, but who have not yet developed symptoms of the disease. These impressive research advances have rapidly taken a newly discovered protein from the laboratory to an approved drug. Further studies unfolding in this field may extend the clinical utility of this new class of therapeutics.

Jodie and Dillon Distel

Participating in Clinical Research To Fight against Type 1 Diabetes

Jodie Distel had just given birth to her son, Dillon, at St. Joseph's Hospital in Denver, Colorado, when she was asked if she would like to participate in something called the Diabetes Autoimmunity Study in the Young, or DAISY. The study, she was told, would initially involve a fairly simple test: Blood from her newborn son's umbilical cord would be screened for genes that could indicate whether he was at high risk for developing type 1 diabetes.

"I didn't know very much about the disease," says Jodie, "but I figured that if taking part in the study might benefit someone else's child or my own son, that it was okay with me." She signed up for the study on the spot.

Within a week after Dillon's birth, Jodie was taken totally by surprise to learn that test results indicated that Dillon was at high risk for developing type 1 diabetes. Later, Jodie recalls, study staff alerted her that it was extremely likely that Dillon would have the disease by the time he was eight years of age. In fact, exactly three days after his seventh birthday, Dillon was formally diagnosed as having the disease.

"I had no idea before taking part in the study that diabetes would be a factor in our lives," says Jodie. Now, looking back, she adds that, "participating in DAISY is probably the best thing I've ever done for Dillon and his future!"

Jodie and Dillon Distel

About Type 1 Diabetes

Type 1 diabetes is an autoimmune disease that destroys a person's ability to produce insulin, a critical hormone the body needs in order to convert sugar from food into life-sustaining energy. Type 1 diabetes most frequently strikes people in childhood, adolescence, or young adulthood. It is characterized by elevated levels of blood glucose, or sugar, which lead to other serious health complications, including eye, kidney, and nerve disease. Adults with type 1 diabetes are also at much greater risk of death from heart disease than adults without diabetes.

Because there is not yet a cure for the disease, people with type 1 diabetes face a daily struggle to manage their disease and prevent complications over the long-term. They must monitor their blood sugar levels and administer insulin via shots or an insulin "pump" every day to enable muscle, fat, and other tissues to absorb sugar from the blood for conversion to energy, and to try to keep blood sugar levels in a stable, healthy range. To help patients, their families,

and people at risk for the disease, the NIH is supporting research on type 1 diabetes with the aim of disease prevention, improved interventions, and, ultimately, a cure.

What Is DAISY?

DAISY is one in a group of epidemiological studies that researchers are pursuing to better understand the underlying causes of type 1 diabetes. The study is based at the University of Colorado Health Sciences Center in Denver. Marian Rewers, MD, the lead investigator for the study, says: "With DAISY, we have two primary objectives. One is to find out what causes [type 1] diabetes; the other is to find ways to prevent it."

To those ends, DAISY researchers are following two groups of children at risk for type 1 diabetes. One group was identified through screening a general population of newborns—which is how Jodie and Dillon got involved in the study. The other group consists of children who have a parent or sibling with type 1 diabetes.

Children who participate in DAISY are followed until they receive a clinical diagnosis of type 1 diabetes or until age 15, whichever comes first. Follow-up includes interviews with the parents to determine a child's diet and exposure to certain viruses, as well as periodic blood tests for three different antibodies against insulin-producing pancreatic islet cells, starting at nine months of age. Like the initial genetic screening, the antibody tests are used to predict risk of developing type 1 diabetes. The presence of antibodies indicates that the autoimmune process has begun. Dillon's blood tests were negative for antibodies against the insulin-producing islet cells until he reached the age of two, at which time he began showing an elevated level of one antibody. Subsequently, his blood was tested more frequently, every 3 to 6 months. At three-and-a-half years of age, he began showing an elevated level of two antibodies. Other markers for diabetes began to change, as well. Over time, Dillon's levels of a marker called HbA1c began to show an upward trend. Finally, his blood sugar levels became elevated. On December 13, 2004, Dillon was diagnosed with type 1 diabetes. He started on a low dose of insulin, and is currently doing very well; as of January 2006, he has never been hospitalized for diabetes-related conditions. With only about one-quarter of the insulin dose it usually takes at his age, physicians are currently able to keep Dillon's levels of the HbA1c marker at a level consistent with improved long-term health outcomes in persons with type 1 diabetes.

Dillon's case appears to support previous observations that early diagnosis helps, to some degree, to preserve the body's own insulin production. This may be in part due to avoiding a condition called diabetic ketoacidosis (DKA). DKA is a dangerous metabolic condition caused by profound insulin deficiency. Prior to diagnosis, many patients with undetected type 1 diabetes will develop DKA, which, if untreated, places them at risk of diabetic coma and death. However, the severe metabolic disturbance of DKA is not only life-threatening, but also further damages any residual insulin-producing cells. Early detection thus helped Dillon to avert both DKA and DKA's negative impact on his already compromised ability to produce insulin—and, by doing so, likely contributed to his need for less aggressive insulin therapy at diagnosis.

"I had no idea before taking part in the study that diabetes would be a factor in our lives," says Dillon's mother, Jodie. Now, looking back, she adds that: "participating in DAISY is probably the best thing I've ever done for Dillon and his future!"

The benefits of early detection and preservation of the body's capacity to produce insulin can last many years. In the landmark Diabetes Control and Complications Trial (DCCT), for example, participants who had preserved insulin secretion not only had better blood glucose control and lower insulin requirements, but also had a 50

percent lower risk of eye complications and a 65 percent lower risk of severe hypoglycemia, or low blood sugar (a risk patients face as a result of insulin treatment).

Thus, early detection of type 1 diabetes can provide both immediate and longer term health benefits. "Dillon is in a much better situation than if we had not participated in the study," says Jodie. In addition to testing a child's blood for antibodies and elevated sugar levels, the families of the children who participate in DAISY are educated about what to expect in the way of symptoms, how to do blood sugar tests at home, and more.

By participating in research studies that follow children from birth who are at high risk for type 1 diabetes, both the parents and children are better prepared if and when a child experiences onset of the disease.

As part of the DAISY research efforts, "one of the best things we do is to educate families, from the time their child's screening indicates high risk, straight through to diagnosis, if that should end up being the case," says Michelle Hoffman, RN, the clinical coordinator for DAISY.

Benefits of the DAISY Study

Since December 1993, the DAISY study has screened more than 33,000 newborns in the Denver, Colorado area for genetic markers that would indicate high risk for type 1 diabetes. Of those, the study has followed more than 2,000 children whose genetic screenings indicated that they were at high risk for developing the disease. Of those, 143 children developed islet cell autoimmunity (ICA)—a condition present in the majority of cases of type 1 diabetes, although people with ICA do not always progress to onset of the disease. Of those 143, 48 have developed type 1 diabetes.[1]

"It should be noted," says Dr. Rewers, "that 90 percent of children in the United States diagnosed with type 1 diabetes are hospitalized at the onset of the disease, and nearly one-third of those enter the hospital with diabetic ketoacidosis (DKA)." According to Dr. Rewers, approximately 100 children die each year of DKA. However, of the 48 children in the DAISY study who went on to develop full-blown type 1 diabetes, only one—an 11-month-old infant—needed to be hospitalized at disease onset.

Therein lies one of the benefits for participants in the DAISY study: By participating in research studies that follow children from birth who are at high risk for type 1 diabetes, both the parents and children are better prepared if and when a child experiences onset of the disease.

Jodie lived for seven years with the hope that Dillon would never be diagnosed with type 1 diabetes. However, when the diagnosis came, she was knowledgeable. "Because of the DAISY program I think Dillon and I were prepared to handle Dillon's being diagnosed, and I think we had to go through far less than any other child and family who do not have the benefit of learning and recognizing early indications of this life-changing disease," she says. "From day one, I was told what symptoms to look for and I mentally prepared myself for this day and how I would help Dillon from that day."

Because diabetes is an insidious disease, "most families are blindsided; they don't know what to look for to recognize onset of the disease," says Dr. Rewers. "When eventually diagnosed, the overwhelming majority of these children end up in the hospital, and many are fighting for their lives—at great emotional expense to themselves and their families, and financial expense to our society." He adds, however, that until researchers can discover and develop prevention strategies to arrest disease onset, they do not currently recommend extending screening programs outside of the research setting.

Research Findings

In addition to refining ways to recognize a genetic predisposition to diabetes and to pursue effective family follow-up, DAISY also has been responsible for a number of significant findings. "For example," says Dr. Rewers, "by closely following these children, we've been able to rule out quite a few environmental factors once suspected as triggers for the onset of diabetes."

DAISY has also opened up new areas for investigation. Researchers, for example, are currently investigating whether the introduction of baby cereals may have something to do with the onset of inflammation in the pancreas that leads to diabetes. "We've discovered through DAISY that if babies at increased risk of type 1 diabetes first eat cereal regularly in their diets before four months of age, or after six months, their risk of islet autoimmunity is four to five times higher than if they begin eating cereal between four and six months of age," says Dr. Rewers. (The current American Academy of Pediatrics recommendation is to breast-feed babies and begin introducing iron-enriched solid foods, such as cereal, beginning at six months of age, if the child is ready.[2]) For children who have a specific genetic marker that is known to strongly predispose individuals to type 1 diabetes, the risk appears to be even greater. According to Dr. Rewers, "these children have an overall increased risk of islet autoimmunity six times higher if fed cereal before age four months, and twelve times higher if cereal is delayed beyond six months, than if they are started on cereal at age four to six months." Research is ongoing to tease out the answers to this and other challenging issues regarding possible causes of type 1 diabetes and factors contributing to its onset.

TEDDY—A Collaborative Effort

In addition to DAISY, other studies have contributed many important insights to advance research on environmental factors in type 1 diabetes. However, there are limitations to smaller studies, such as the number of patients that can be recruited in a given location.

To overcome these limitations, the NIH spearheaded the launch of a long-term, international, collaborative effort to identify environmental triggers of type 1 diabetes. This effort, begun in 2002, is called "The Environmental Determinants of Diabetes in the Young," or TEDDY. Funded by the Special Statutory Funding Program for Type 1 Diabetes Research (see *http://www.T1Diabetes.nih.gov*), TEDDY consists of six centers in the U.S., Finland, Sweden and Germany. The creation of the TEDDY consortium allows for a coordinated, multidisciplinary approach; collection of data and information in a standardized manner; greater statistical power than can be achieved in smaller studies; and the creation of a central repository that includes data and biological samples for use by the scientific community.

Researchers participating in TEDDY—including the Denver investigators who have conducted DAISY—are recruiting newborns who are genetically predisposed to developing type 1 diabetes. They are screening newborns from the general population, as well as newborns who have parents or siblings with the disease. The children will be followed until they are 15 years old or until they develop islet autoimmunity or type 1 diabetes. This long-term study will amass the largest data set and samples on newborns at risk for type 1 diabetes anywhere in the world.

"The more brain power contributing to this effort, and the better we can coordinate our work and findings, the greater the chances of our discovering ways to more quickly develop prevention strategies for type 1 diabetes," says Dr. Rewers.

TEDDY was established by the NIDDK, the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, the National Institute of Environmental Health Sciences, the Centers for Disease Control and Prevention, the Juvenile Diabetes Research Foundation, and the American Diabetes Association.

PATIENT PROFILE

"The more brain power contributing to this effort, and the better we can coordinate our work and findings, the greater the chances of our discovering ways to more quickly develop prevention strategies for type 1 diabetes," says Dr. Rewers.

[1]*These numbers are current as of December 2005.*
[2]*http://aappolicy.aappublications.org/cgi/content/full/pediatrics;115/2/496*

TEDDY is currently enrolling patients. TEDDY enrollment sites in the United States are located in Georgia, Florida, Colorado and Washington state. For more information on enrolling in TEDDY, please see:
http://www.niddk.nih.gov/fund/diabetesspecialfunds/t1d_ctcr/study.asp?StudyID=121

PATIENT PROFILE

Todd Hutchinson

Dealing with Type 2 Diabetes

Fourteen-year-old Todd Hutchinson and his mother, Lisa, live in Tahlequah, Oklahoma, capital of the Cherokee Nation. As capital cities go, Tahlequah, a one-hour drive east of Tulsa, is quite small, with a population of about 15,000. American Indians, including Todd, his mother, and other Cherokee descendants, make up a large percentage of the population. Despite Tahlequah's size, "there are many people with diabetes here," says Lisa, whose own family reflects the severity of the problem. Lisa, Todd, Lisa's father, her sister, as well as other extended family members, including Todd's step-grandmother, have been diagnosed with type 2 diabetes.

According to statistics compiled by the NIDDK and other agencies of the Department of Health and Human Services, American Indians and Alaska Natives are more than twice as likely to have diagnosed diabetes as non-Hispanic whites of similar ages.[1] More disturbing is the fact that, until recently, type 2 diabetes was rarely diagnosed in children and adolescents. However, type 2 diabetes is now diagnosed more frequently within these age groups, particularly among American Indians, African Americans, and Hispanic/Latino Americans. Genetic susceptibility, reduced physical activity, and obesity are viewed as major contributors to this alarming trend—and to personal stories. For example, Todd weighed more than 250 pounds and led a relatively sedentary lifestyle prior to being diagnosed with type 2 diabetes in April 2005, at age 13.

Todd Hutchinson, and his mother, Lisa

Type 2 diabetes is being diagnosed more frequently in children and adolescents, particularly among American Indians, African Americans, and Hispanic/Latino Americans.

Todd is an extraordinarily bright, articulate, studious young man, who aspires to become a physician one day. A couple of months after his diagnosis, he was enrolled in the study of "Treatment Options for Type 2 Diabetes in Adolescents and Youth," commonly referred to as the TODAY study. Begun in March 2004, and funded by NIDDK, TODAY is comparing treatments for type 2 diabetes in children and teens in 13 medical centers and their affiliated sites across the United States. The aim of the study is to identify the best therapeutic strategies to combat this disease in young people. According to Todd and his mother, Todd's participation in the TODAY study has greatly benefited both of them.

PATIENT PROFILE

About TODAY

Participants in the TODAY study are young people with type 2 diabetes, a disease characterized by the body's resistance to the action of insulin. The trial is testing two drugs that help to fight the disease by increasing the body's sensitivity to the insulin it produces. In addition, the trial is studying intensive lifestyle changes aimed at lowering weight by cutting calories, improving nutrition, and increasing physical activity.

Young people interested in joining the TODAY study first complete a two-part screening process to determine their eligibility, during which they receive a comprehensive program of standard diabetes education that includes important information about nutrition and physical activity. Following the screening process, eligible participants are then enrolled in a treatment group. The TODAY study has three treatment groups. Participants in one group are provided with the drug metformin alone. Metformin is the only oral drug approved by the Food and Drug Administration to treat type 2 diabetes in children. In the second group, participants are provided with metformin in combination with rosiglitazone, another promising oral drug currently approved only for adults. Participants in the third group receive metformin and also participate in a family-based behavioral weight loss program that focuses on cutting calories and increasing physical activity. Notably, TODAY is the first clinical study to look at the health effects of intensive lifestyle change in youth with type 2 diabetes. It is based upon previous studies in adults, which show that relatively modest weight loss and increased physical activity can substantially reduce blood sugar levels.

Because type 2 diabetes was previously very rare in children, there is very little information on how best to treat it. By supporting major research studies that are aimed at developing optimal treatment of type 2 diabetes in children—like the TODAY trial—the NIDDK hopes to ameliorate this disease and its complications in this most vulnerable population.

Todd was randomly assigned to one of the TODAY study's three treatment groups. Researchers are planning to enroll 750 children and teens, ages 10 to 17, who have been diagnosed with type 2 diabetes within the past 2 years. The trial will last approximately 5 years and is expected to answer urgent questions about which therapy is most effective for treating type 2 diabetes in young people.

Why Early Intervention Is Important

The longer a person has diabetes, the greater the chances he or she will sustain serious damage to blood vessels of the eyes, nerves, kidneys, and heart. Todd's grandfather, for example, had his foot amputated and later became legally blind as a result of complications from diabetes. This aspect of diabetes makes the growing burden of type 2 diabetes in children particularly alarming, because children with this diagnosis have a greater statistical chance of developing medical complications during their lifetimes. Therefore, the prevention of type 2 diabetes in youth is a primary public health goal. However, optimizing type 2 diabetes treatment alternatives is equally critical in order to forestall the onset of complications in children who already have the disease.

One Smart Young Man

It doesn't take long to figure out that Todd Hutchinson, now 14, is a smart and highly motivated young man. In addition to being an avid reader, Todd takes part in extracurricular school activities, such as the Esperanto Club, and fiercely competes in the "tournament of champions" academic competitions in the state of Oklahoma. Todd says that he likes to look up things he doesn't know about on the Internet. So, he investigated when he began experiencing excessive thirst, unexplained weight loss, and extreme hunger in the middle of the night, as well as very dry skin, sleepiness, blurred vision and a tingling sensation in his hands. "I went on the Internet and learned that I was manifesting all the classic symptoms of type 2 diabetes," he says in a sophisticated voice that belies his relatively young age. His mother

adds that: "Todd didn't want to accept the fact that he might have type 2 diabetes." The tipping point came when Todd had surgery for an ingrown toenail and it wouldn't heal. "My mom took me to the doctor, and he found that my blood sugar levels were running between 700 and 1,000 [milligrams per deciliter]," says Todd. The normal range for blood sugar levels for people without diabetes is about 10 times lower. Todd remembers that: "The doctor was amazed that nothing bad had happened to me, and told me and my mom that I could have easily gone into a diabetic coma and died, and that I was very lucky not to have had any serious repercussions."

Todd later enrolled in the TODAY study on the recommendation of his physician. "I enjoy being in the study," says Todd. "It's a little bit of work, but it's well worth it."

Taking Part in the TODAY Study

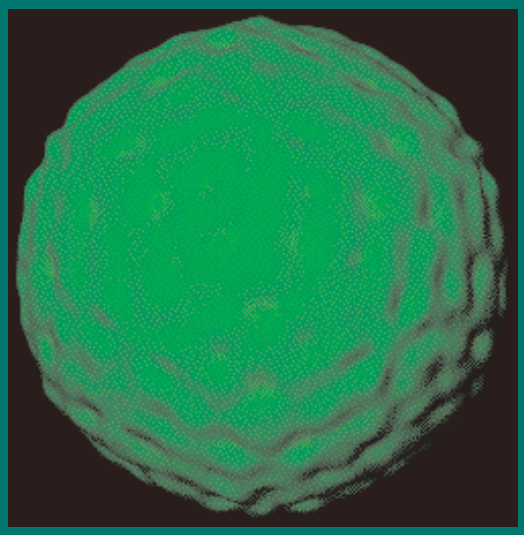
As a study participant, Todd's health records and other personal information were collected. Todd and his mother also went through an intensive education program that included information about diabetes, the impact of lifestyle and diet on the disease, and the steps that study participants and their families can expect to follow over the next two to five years of the study, including the frequency with which Todd's blood sugar levels must be checked. Todd also keeps a daily journal of his food intake, exercise activities, and more. He says that: "One of my responsibilities is to bring my glucose meter with me wherever I go." Todd is aware of the potential consequences of his disease, and says, "I have a group of friends who help me out a lot, and I've told them what to look for if my sugar gets too high or too low."

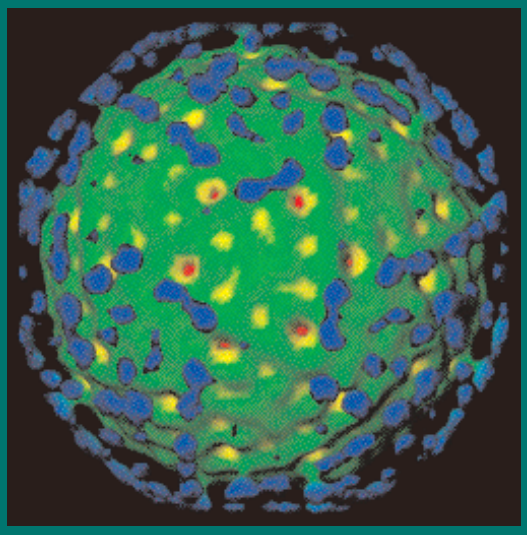
As part of the study, Todd has a personal activity leader, or PAL, who comes to his home once a week to monitor his progress and to continue educating Todd and his mother about lifestyle and eating habits and how they affect diabetes. Todd, for example, has gone from eating cheeseburgers and pizzas and drinking two-to-three liters of soda a day ("we call it pop, here," says Todd), to consuming more healthful foods, including vegetables. "My diet has changed tremendously," he says. "I like stir fried vegetables, and one of my favorites is broccoli."

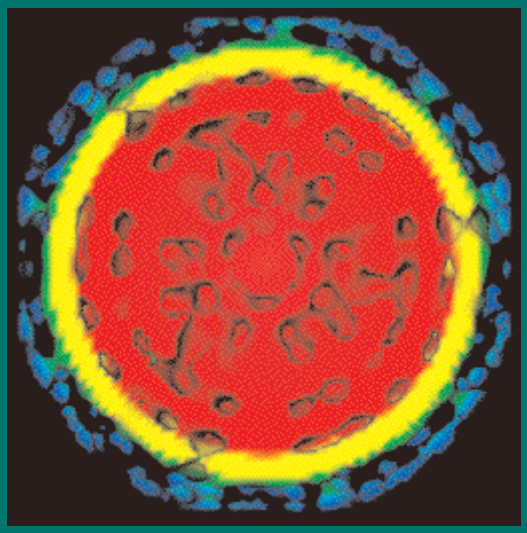
Because he wants to adhere to all the expectations of the study, Todd is engaging in more physical activities, as well. "I ride my bike 10 or more miles a week and I often go on family hikes through the woods." Nearly every day he and his mother walk two miles around a local high school track. As a result, in a matter of months, Todd's weight has gone from 250 pounds down to 171 pounds. "My goal is 160 pounds, which would be a good weight for me," he says. His mother, who was diagnosed with type 2 diabetes seven years ago, adds that the exercising she does with Todd and the information she has received about diabetes as a result of the TODAY study have also benefited her health.

Exercise, change of diet, and weight loss are helping Todd keep his glucose levels in a healthy target range. "The TODAY study has really changed Todd's life," says his mother. Todd agrees. "When I was first diagnosed with type 2 diabetes, I was a bit worried, but I told myself 'you've got it and you can't change that, so you had better deal with it.' The TODAY study has helped me do that. I just hope they find a cure for type 2 diabetes so that kids like me can live our lives as normal kids, without fear of going blind or losing a limb." In the meantime, Todd wants to be part of the solution. "I want to become a physician so I can help other people with diabetes. It's a great ambition of mine." And given his determination, it's surely an ambition this engaging young man with deep American Indian roots can realize.

[1] *National Institute of Diabetes and Digestive and Kidney Diseases. National Diabetes Statistics fact sheet: General information and national estimates on diabetes in the United States, 2003. Bethesda, MD: U.S. Department of Health and Human Services, National Institutes of Health, 2003. Rev. ed., 2005.*







At left are reconstructed images of a recombinant hepatitis C virus (HCV)-like particle that was produced in a cell culture system. These images are three-dimensional representations of the HCV particle at high resolution. Visualizing the virus in this way enables researchers to understand how it interacts at the molecular level with compounds important in fighting against HCV infection, such as antibodies. Top: The exterior surface of the HCV particle. Middle: The HCV particle labeled with antibodies (shown in blue), which bind to proteins on the surface of the viral coat. Bottom: A cross-sectional view of the antibody-labeled HCV particle shown in the middle panel. The internal structure of the virus is shown in red. For more information about recent exciting advances in HCV research, see the Feature entitled, "Small Cells Yield Big Breakthrough in Hepatitis C Research," in this chapter.

Images courtesy of Dr. T. Jake Liang, Chief, Liver Diseases Branch, NIDDK Division of Intramural Research.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract—such as irritable bowel syndrome and inflammatory bowel disease—exact a significant toll on many Americans each year. NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.

Several types of liver disease have serious adverse impacts on health, and some can lead to complete liver failure. Some liver diseases primarily affect children—such as biliary atresia, a progressive inflammatory liver disease—while others more commonly affect adults—such as nonalcoholic steatohepatitis (NASH). Some are caused by viral infection—such as hepatitis C—while others arise from diverse factors such as autoimmune reactions, genetic mutations, drug toxicity, and other, unknown triggers. A functioning liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. The number of livers available from deceased donors is limited, and research is of critical importance to identify and treat liver disease, preserve liver function in people with liver disease, and explore treatment options beyond cadaveric liver transplants.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. While multiple factors contribute to obesity, caloric intake clearly plays a key role in weight gain. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacologic agents to promote safe, long-term weight loss. Investigators are also continuing behavioral research to help people achieve healthy lifestyles that include increased physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the next chapter.)

Intestinal disorders 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.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued identification of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences.

The microorganisms that inhabit the gastrointestinal tract are powerful players in maintaining or tilting the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with cells of their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract. Some digestive diseases can be triggered by the body's

reaction to certain foods. In individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their healthcare 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.

LIVER DISEASE RESEARCH

A New Model System To Study Hepatitis C: Hepatitis C virus (HCV) is a major cause of liver disease worldwide. Current therapies for HCV are not optimally effective and a vaccine is not yet available to prevent the disease. Clinical progress has been hampered because the virus grows poorly in culture and does not produce infectious viral particles. To solve this research problem, scientists recently designed a molecular HCV replication system that is capable of producing viral particles *in vitro*. HCV was detectable inside of the cells and mature viral particles were found in the culture medium. These particles were able to infect cultured cells and an animal model of HCV. With this advance, researchers will be better able to study the life cycle and biology of this virus, and to test antiviral compounds as potential therapies for the liver disease it causes.

For more information about exciting research advances in the study of hepatitis C, see the Feature in this chapter.

Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, and Liang TJ: Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 11: 791-796, 2005.

NAFLD, a Disease Without a Treatment: Recently, a clinical trial was launched to study fatty liver disease in children. The trial, termed TONIC for "Treatment of Nonalcoholic Fatty Liver Disease (NAFLD) in Children," is part of a larger NIDDK initiative, the Nonalcoholic Steatohepatitis Clinical Research Network, which is also supporting adult studies of this disease. The major characteristic of NAFLD, as its name implies, is the accumulation of fat in the liver. If inflammation and liver injury are also present, this condition is known as nonalcoholic steatohepatitis or "NASH." NAFLD is very similar to the better known condition of alcoholic liver disease; however, it is seen in children and in adults who drink little or no alcohol. NAFLD is associated with overweight and obesity and occurs in a high proportion of persons with diabetes. The rate of NAFLD in children has increased sharply and appears to correlate with the rapid rise recently in the rate of childhood obesity.

Although most children with this disease feel well and do not experience symptoms, researchers are concerned that, as they grow into adulthood, the disease may progress, increasing their risk for cirrhosis, particularly if they drink alcohol or contract viral hepatitis. Currently, there are no safe or effective drugs recommended to treat children or adults with NAFLD. If cirrhosis occurs, liver damage can be so severe that a liver transplant is the only treatment option.

The NIDDK and investigators involved in the TONIC trial hope that it will uncover the underlying conditions that contribute to the development and progression of NAFLD, as well as test the safety and effectiveness of new treatments. Results from previous small pilot studies using antioxidants, such as vitamin E, or the insulin-sensitizing drug metformin showed that these agents may improve the condition of patients with NAFLD and may delay or possibly prevent the progression to more serious liver disease. Extensive safety and efficacy data already exist on the use of metformin for the treatment of type 2 diabetes in children.

The TONIC trial will enroll 180 girls and boys, ages 8-15, with NAFLD. Because of the association of NAFLD with obesity, 90 percent of the children enrolled in the trial are expected to be obese. However, children with other liver diseases or diabetes will be excluded from the trial. This trial has three arms in that participants will receive either vitamin E, metformin, or a placebo, for a period of two years.

As the first randomized, controlled clinical trial for children with NAFLD, TONIC is expected to provide a platform to conduct rigorous studies on how safe and

effective vitamin E and metformin are as treatments. Data from this trial, along with the adult trials supported by the NIDDK through the NASH Clinical Research Network, will contribute to greater understanding of NAFLD, advance treatment options, and improve outcomes for children and adults with this disease.

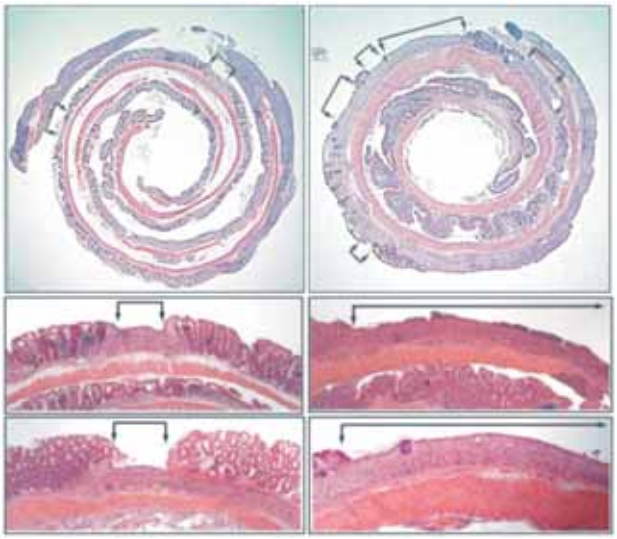
Molecular Factors Underlying Liver Development: Understanding how a single fertilized egg develops into a complex, multicellular organism is one of the most fascinating questions in all biology. Scientists studying liver development have found that the *Foxa1* and *Foxa2* genes play an important role in the developing liver. It is thought that embryonic liver development proceeds in a two-stage process whereby factors first make the tissue "competent" to respond to subsequent organ-specific signals that direct tissue differentiation into specific organs. To explore this area, investigators derived a strain of mice lacking the *Foxa1* gene entirely and lacking the *Foxa2* gene in endoderm—the embryonic tissue that gives rise to the liver. Embryos in which these genes were made non-functional (knocked out) were smaller than normal counterparts and failed to develop an embryonic liver. When normal mouse endoderm is grown in culture in the presence of certain growth factors, the tissue begins to express proteins characteristic of mature liver. In contrast, endoderm from *Foxa1/Foxa2* knockout embryos grown under these conditions did not express liver-specific proteins, suggesting that this tissue is not able to respond to the growth factors. This observation suggests that the proteins encoded by *Foxa1* and *Foxa2* genes act early in liver differentiation as competence factors that render the tissue able to respond to subsequent signals. This experimental system may be very useful in the study of organ development, because the endoderm gives rise to many tissues, including the gut, pancreas, thyroid, and lungs.

Lee CS, Friedman JR, Fulmer JT, and Kaestner KH: The initiation of liver development is dependent on Foxa transcription factors. Nature 435: 944-947, 2005.

New Imaging Methods for Hepatic and Renal Fibrosis: A common problem faced by those studying diseases of the liver and kidney is how to monitor disease progression, particularly how to assess the fibrosis that occurs in these organs as part of the disease process. In fiscal year 2007, the NIDDK plans to solicit research applications to develop non-invasive imaging methods that will allow early detection of renal and hepatic fibrosis and will facilitate monitoring of disease progression. An essential component will be cooperation between investigators who develop cutting-edge imaging methods and investigators who are familiar with the disease issues relevant to fibrosis. This effort will have significant impact on the ability of investigators to pursue novel therapies for many kidney and liver diseases, because it promises to provide a cheaper and more reliable measure of outcomes than current methods. The NIDDK also has plans to pursue a Program Announcement, to be addressed through the Small Business Innovation Research program, that will reinforce and complement the studies funded through the research solicitation on fibrosis imaging.

NEW MODELS SHED LIGHT ON INFLAMMATORY BOWEL DISEASE

Insights into Crohn's Disease from Two New Mouse Models: Crohn's disease (CD) is a chronic inflammatory bowel disease thought to arise from a combination of genetic susceptibility and environmental factors. Previous studies have identified mutations in the *Nod2* gene as playing an important role in the development of CD in humans. To better clarify the role of *Nod2*, researchers have developed two new mouse models of the disease. One group of researchers generated mice lacking the *Nod2* gene entirely. The animals were resistant to infection from bacteria introduced intravenously, but were more susceptible than normal animals to infection resulting from orally-administered bacteria. This finding highlights the protective role *Nod2* plays in fighting bacterial infection in the gut. Another group of researchers generated mice in which the normal *Nod2* gene was replaced with the most common mutant form seen in human CD. A chemical agent that damages the cells lining the intestine caused greater weight loss, increased mortality, and more severe colonic ulcerations in animals with the mutant *Nod2* gene than in normal animals. This ulceration could be minimized by the co-administration of antibiotics, suggesting that the increased damage resulted from interactions among the chemical, the bacteria present in the intestine, and the *Nod2* gene. Immune cells cultured from mice with the mutant *Nod2* gene showed increased production of a number of pro-inflammatory proteins including one of the interleukins (IL-1beta). *Nod2* mutant mice treated concomitantly with an agent that blocks IL-1beta activity and the chemical agent



Scientists use animal models to study diseases and evaluate potential new therapeutic approaches. Research has indicated that mutations in the *Nod2* gene likely play an important role in the development of Crohn's disease, a chronic inflammatory bowel disease, but the precise role of these mutations is unknown. Shown above are histological sections (at varying magnifications) of large intestine taken from normal mice (left) and from mice with mutant *Nod2* genes (right) after they had been treated with a chemical that causes ulcers. The arrows in the panels denote the borders of ulcers. Mice with the mutant gene exhibit substantially more inflammation and ulceration in their intestines, which provides further experimental evidence that this gene contributes to inflammatory bowel disease.
Image courtesy of Dr. Martin F. Kagnoff and reprinted with permission from Maeda et al. Science 307: 734-738. Copyright 2005 AAAS.

showed improvements with respect to weight maintenance and colonic ulcerations, compared to mutant animals not receiving the blocking agent. Together, these studies identify important new roles for *Nod2* in the development and progression of CD.

Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, and Flavell RA: Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307: 731-734, 2005.

Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, Eckmann L, and Karin M: Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science 307: 734-738, 2005.

BACTERIA IN THE GUT: CONTRIBUTION TO SENSITIVITY TO RADIATION

Important Insights into the Causes of Side Effects of Radiation Therapy: Scientists have recently shed new light on a very old treatment for cancer. Radiation therapy (sometimes called radiotherapy) was first used to treat cancer more than 100 years ago, and it remains a critical part of the treatment approach for almost half of cancer patients. Radiation kills living cells, but is particularly damaging to cells that divide frequently, such as cancer cells. However, the damage to other tissues caused by radiation therapy can limit the usefulness of the approach. Scientists have recently found evidence that the degree of radiation sensitivity in the intestine may be influenced in part by the trillions of bacteria that reside there. The researchers showed that mice raised in a germ-free environment are more resistant to the side effects of radiation than mice grown in the conventional way. Bacteria play a valuable role in the digestive process for all mammals, including people, but scientists are just beginning to appreciate the complex physiological interaction between animals and the bacteria that reside in their bodies. One effect the microbes have is that they decrease the expression level of a particular protein that may help protect against radiation. This finding raises the possibility that radiation therapy might be enhanced if physicians are able to either manipulate the microbial environment of the intestines or influence the expression level of this protein. Physicians may one day be able to reduce side-effects and improve survival in patients receiving radiation therapy.

Crawford PA, and Gordon JI: Microbial regulation of intestinal radiosensitivity. Proc Natl Acad Sci U S A 102: 13254-13259, 2005.

LINK BETWEEN FASTING AND A RARE DISEASE

A Protein that Couples Nutritional Status to Heme Synthesis: Researchers studying a protein that helps regulate metabolism during fasting have found an important link to understanding porphyria. Porphyria is a rare but serious condition characterized by acute attacks of abdominal pain, severe psychiatric and neurological problems, and sensitivity to sunlight. The attacks occur in susceptible people as a result of fasting or of taking certain

drugs or hormones. Susceptibility is caused by any of several rare mutations that interfere with biosynthesis of an essential iron-containing compound called "heme." Heme is a central constituent of hemoglobin, a major component of red blood cells, required for ferrying oxygen throughout the body. Heme is also used in every cell of the body as part of the energy-utilization machinery; in the liver, where it plays a vital role in neutralizing toxins, as well as many drugs; and in a variety of other proteins throughout the body. Unfortunately, heme is actually toxic when there is too much of it around, so its production is tightly controlled. Researchers studying PGC-1alpha, a protein that helps the body respond to cold temperatures by boosting energy utilization, noted that the protein is also up-regulated in the liver in response to fasting. Because fasting is also known to trigger attacks of porphyria, the scientists tested whether PGC-1alpha is also responsible for up-regulating heme biosynthesis—and found that it is. These results not only help explain what triggers porphyria attacks, they also clarify how the attacks can be blunted by treatment with glucose. They represent another piece in the complex puzzle of how the body maintains energy balance.

Handschin C, Lin J, Rhee J, Peyer AK, Chin S, Wu PH, Meyer UA, and Spiegelman BM: Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha. Cell 122: 505-515, 2005.

NATIONAL COMMISSION ON DIGESTIVE DISEASES

On September 20, 2005, the NIH Director announced the establishment of the National Commission on Digestive Diseases, which will work to improve the health of the nation through advancing digestive diseases research. The Commission is responsive to the mutual interest in this area shared by the Congress, the NIH, and the research community. Within the NIH, the NIDDK is providing leadership and support for the Commission, which will be active for two years as it develops a long-range research plan.

As part of its charge, the Commission will assess the state-of-the-science in digestive diseases and the related NIH research portfolio, in order to identify research challenges and opportunities for inclusion in the plan. The resulting 10-year plan will guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases. The Commission's efforts will benefit from the diverse expertise of its members, representing the NIH and other Federal health agencies, the academic and medical research and practice communities, and the patient advocacy community. Additional information on the Commission can be found on its website: *http://NCDD.niddk.nih.gov.*

PUTTING A SPOTLIGHT ON CELIAC DISEASE

Celiac disease was once thought to be a rare disease, but it is now believed to affect as many as three million Americans. This disease, which often goes undiagnosed, results from an immune response mounted by cells in the intestinal tract to the common protein gluten, which is found in grains such as wheat, barley and rye. Celiac disease has strong genetic ties. Currently, the only treatment for celiac disease is a gluten-free diet, which results in remission for most affected individuals.

In June 2004, a Consensus Development Conference on Celiac Disease, sponsored by the NIDDK and the Office of Medical Applications of Research, was held at the NIH. The meeting focused on currently available data regarding the awareness, diagnosis, and management of celiac disease. In a Consensus Statement, the conference panel concluded that heightened awareness of this disease was imperative. It recommended that the NIDDK lead an educational campaign for physicians, dietitians, nurses, and the public about celiac disease.

In response to the Consensus Statement, the NIDDK's National Digestive Diseases Information Clearinghouse (NDDIC) is developing a Celiac Disease Awareness Campaign designed to inform and educate healthcare professionals and the public about the disease. An *ad hoc* committee—with representatives from the medical and health care fields, interest groups, and the clearinghouse—held a conference call to discuss the best strategies to raise awareness of the disease.

To supplement these strategies, research was conducted with health care professionals to determine the level

of awareness of celiac disease, to identify barriers to diagnosing celiac disease, and to elicit ideas to heighten awareness of celiac disease. This research included focus groups with a total of 72 primary care physicians in four states. Results from the focus groups indicated that an awareness campaign will need to center on the prevalence of celiac disease, some of the less-well-known, non-gastrointestinal symptoms, and the long-term consequences for patients who are not diagnosed.

As part of the new campaign, an updated version of the Celiac Disease Fact Sheet is now available on the Clearinghouse website *http://digestive.niddk.nih.gov*. Copies of the June 2004 Consensus Development Conference Statement also are available on the site.

FEATURE

Small Cells Yield Big Breakthrough in Hepatitis C Research

The hepatitis C virus (HCV) is one of at least five hepatitis viruses (hepatitis A to E viruses) that cause liver disease in humans. However, HCV stands out as the main cause of chronic hepatitis, cirrhosis, and liver cancer in the United States, and the major reason for liver transplantation in American adults.[1, 2] *Obtaining fundamental knowledge of HCV has been a daunting task for researchers, due to a lack of useful or convenient cell culture or animal models of HCV infection. The challenge of developing better HCV infection models to put in the hands of researchers eager to address persistent questions has been a long-standing focus of Dr. T. Jake Liang and his group in the NIDDK Liver Diseases Branch. Recently, their efforts have come to fruition in the form of a major breakthrough in the field—a cell culture system for HCV that promises to shed light on viral strategies for causing disease, how to counter and prevent them, and to generally accelerate the rate of discovery.*

State of Knowledge on Hepatitis C

Population-based surveys in the U.S. estimate that 2.7 million adults harbor HCV RNA in their blood, indicating a chronic HCV infection.[3] The virus is spread mainly through contact with infected blood due to needle sharing or receipt of contaminated blood products. However, it can also be transmitted through sex with an infected partner, occupational exposure to infectious blood, organ transplantation from an infected donor, unsafe medical practices, or birth to an infected mother. While a therapy for hepatitis C exists—typically a combination of antiviral drugs called peginterferon/interferon and ribavirin—these agents often cause major side effects and are effective in only about half of patients. No vaccine exists to prevent the spread of hepatitis C. While much is known about the serious public health threat posed by HCV, gaps in knowledge persist regarding its means of infecting cells and causing disease—important pieces of the puzzle for developing more effective approaches to treatment and prevention. For other types of viral hepatitis, the availability of good cell and animal models has accelerated the pace of discovery in recent years, leading to such advances as the development of safe and effective vaccines for hepatitis A and B, and the discovery of new drugs to treat hepatitis B. In contrast, the pace of research on HCV has been relatively slower since its identification in 1989. An improved working knowledge of HCV and its infection strategies in basic research models could enable the development of better treatments and the elusive HCV vaccine.

The Inimitable Hepatitis C?

In order to understand the pathogenic impact and uncover therapeutic or preventive weaknesses of the hepatitis C virus, researchers must understand its structural composition and how it infects the liver cells in which it causes disease. The basic components of HCV have been identified; from the outside working in, the virus is made up of two envelope proteins, a core or "nucleocapsid," and a positive-strand RNA genome. The RNA genome of HCV can vary, with at least six known genetic variants or "genotypes" of HCV. HCV genotypes 1-6 appear in different frequencies in infected populations throughout the world, with genotype 1 being the most common in the U.S. and worldwide, as well as the most difficult to treat using standard antiviral therapy. Once a person is exposed to HCV through one of the transmission routes mentioned previously, the full viral life cycle involves: (1) entry of the virus into the host cell (typically a hepatocyte, a liver cell); (2) production of structural proteins and enzymes; (3) replication of its RNA genome; (4) assembly; and (5) release of new

viruses from the infected cell, which then repeat the cycle by infecting neighboring, healthy cells.

HCV infection has proven particularly difficult to mimic in cell culture and in the small animal models typically used by basic researchers, for reasons that are currently unknown. What little is known to date of HCV's structure and life cycle of infection is the result of years of research using available, but sub-optimal cell culture and animal models for studying HCV. The chimpanzee model is widely considered to be the best animal model of HCV infection. However, research using this model instead of the mice or rats used most frequently for laboratory research is more costly, time-consuming, and inconvenient, due to such issues as limited access to research animals. As an alternative to animal models and in order to observe and manipulate viral behavior in a simplified system, researchers struggled for years to create an efficient cell culture model for HCV. An important advance was the development of the HCV "replicon" system, which enabled study of viral replication. Yet, even this model could not recreate the entire viral life cycle to yield mature viruses. The elusive "holy grail" in basic research on HCV was an efficient and reproducible cell culture system capable of supporting all stages of the viral life cycle to produce infectious hepatitis C viruses.

Cracking the Case—A Cell Culture Model for HCV

At the NIDDK, Dr. Liang's group of researchers, including Dr. Theo Heller, Dr. Satoru Saito and Dr. Takanobu Kato, worked for several years to develop an innovative solution to the lack of a useful cell culture model for HCV. They pursued multiple research approaches in parallel to address the problem from several fronts. Their primary focus was on creating a DNA expression "construct" that would produce the complete HCV RNA genome, including its complex end structures that are important for HCV replication and production, within the liver cell. To do this, they obtained HCV RNA from a patient with HCV genotype 1 and "reverse transcribed" the RNA into DNA, to which they added special sequences coding for "ribozymes," enzymes that cut RNA at precise locations. When transferred into human liver cells in culture and transcribed, the construct not only produced the HCV RNA genome, but also added the ribozymes to its ends, which cut the viral RNA to recreate the unique structures at the ends of the genome. After delivering the HCV RNA genome into the liver cells, Dr. Liang's group showed that their HCV-ribozyme cell culture system was capable of producing viral RNA and proteins, as well as assembling and releasing full viral particles. The publication of these findings in the *Proceedings of the National Academy of Sciences* in February 2005 signaled a groundbreaking achievement—the first cell culture system capable of producing high levels of complete HCV particles.[4] Soon after this publication, the group went on to show that the particles of HCV produced by this system are capable of infecting other healthy liver cells, indicating that the system can be used to study the complete life cycle of HCV and to produce infectious virus.

While the HCV-ribozyme cell culture system was in development, Dr. Liang also collaborated with Japanese and German researchers on a different, but complementary research approach to creating a cell culture model for HCV. For these studies, the researchers utilized a unique strain of virus isolated from a Japanese patient infected with a genotype 2 HCV. They introduced the HCV RNA genome directly into human liver cells in culture. In this system, they observed robust replication of the RNA and production of viral particles, which were secreted from the cells and, most importantly, found to be infectious in both healthy liver cells in culture and when injected into healthy chimpanzees. When these findings were published in *Nature Medicine* in July 2005, they represented the first public demonstration of a cell culture system capable of producing HCV particles that were shown to be infectious.[5]

Since the announcement of these two parallel research advances to develop an HCV cell culture

system, Dr. Liang's group has combined the two approaches. They introduced the robust HCV genotype 2 strain from the Japanese patient into the HCV-ribozyme construct system, again demonstrating successful production of HCV particles. The advantage of using the HCV-ribozyme construct system, which uses DNA to deliver the HCV RNA genome to liver cells as opposed to directly delivering viral RNA, is that DNA is more convenient for researchers to handle, assay, and store. Additionally, the DNA-based construct could be used in future experiments to generate continuous cell lines based on DNA's ability to integrate into the cell's genome.

Collaboration and friendly competition within the international research community drove these advances and helped to dispel skepticism that the "holy grail" of an efficient cell culture system for HCV could not be found. Around the time that Dr. Liang and his collaborators in Japan and Germany announced their success in developing a cell culture system using the robust HCV genotype 2 strain, two other American research groups, one of which received partial support from the NIDDK, published similar results using the same HCV strain. Recently, other groups have also demonstrated the success of HCV DNA expression culture systems like the HCV-ribozyme system developed by Dr. Liang's group.

New Research and Clinical Possibilities for Hepatitis C

The number of potential uses for the cell culture systems developed by Dr. Liang's group, and through his collaborations with Japanese and German researchers, is limited only by scientists' imaginations. Planned applications range from basic research on how HCV causes disease to tools for drug development.

Because Dr. Liang's group used a genotype 1 HCV in their initial experiments to test the HCV-ribozyme culture system, these studies have implications for the widest number of people with hepatitis C, many of whom do not respond well to standard antiviral therapy. However, Dr. Liang's group also plans to create HCV-ribozyme constructs of all HCV genotypes, using viruses isolated from patients, in order to study the basis for the unique pathology and response to treatment associated with different HCV genotypes. Plans to use the HCV-ribozyme construct to develop stable cell lines that continuously produce HCV would save researchers from having to reintroduce the virus for each experiment and would facilitate comparison of findings across research groups. The production of high levels of HCV in the HCV-ribozyme cell culture model is even enabling highly detailed views of the 3-dimensional structure of the virus at the level of its individual molecules; this knowledge will inform researchers about how HCV interacts with host cells and antibodies.

Additionally, these cell culture systems can be used to screen new drugs for their effectiveness in treating HCV infection. Dr. Liang's group has already tested a standard treatment for hepatitis C, interferon, on the cell culture system and confirmed that it is effective in the system at fighting the virus. Another attractive research direction for this system is to develop an experimental HCV vaccine that would be effective against the widest variety of HCV genotypes found throughout the world.

While these cell culture models provide researchers with a simplified system in which to study HCV replication and infection, animal models are still key to understanding how HCV behaves within a whole organism. Small animal models of HCV infection are needed to study the viral life cycle, host immune response, natural history, and response to experimental therapy and vaccines. Dr. Liang's group hopes to address this need by using the same HCV-ribozyme construct to create transgenic mouse models that have the construct in their DNA and, therefore, naturally produce HCV. These mouse models would enable researchers to study the effect of specific HCV genotypes and experimental therapies to treat hepatitis C or to prevent its progression to diseases such as liver cancer. This research in animal models would complement ongoing clinical trials on

treatment and prevention of chronic hepatitis C progression such as the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis or "HALT-C" trial supported by the NIDDK.

Dr. Liang is sharing his constructs, cells, and other materials with the research community to enable pursuit of the host of experimental possibilities opened up by the availability of a cell culture system for HCV. He hopes that other researchers—either those already studying HCV or who were reluctant to do so due to a lack of research tools—will also use these small cells to think big…and find answers to many lingering questions about HCV.

[1] *Vong S and Bell BP. Chronic liver disease mortality in the United States, 1990-1998. Hepatology 39: 476-483, 2004.*

[2] *2003 Annual Report of the U.S. Organ Procurement and Transplantation Network (OPTN) and the Scientific Registry of Transplant Recipients (SRTR): Transplant Data 1993-2002. Department of Health and Human Services, Health Resources and Services Administration, Office of Special Programs, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor, MI. Available online at http://www.optn.org/AR2003/default.htm Accession date: December 19, 2005.*

[3] *Centers for Disease Control and Prevention, Division of Viral Hepatitis, "Disease Burden from Viral Hepatitis A, B, and C in the United States," 2003.*

[4] *Heller T, Saito S, Auerbach J, Williams T, Moreen TR, Jazwinski A, Cruz B, Jeurkar N, Sapp R, Luo G, Liang TJ. An in vitro model of hepatitis C virion production. Proc Natl Acad Sci U S A 102: 2579-2583, 2005.*

[5] *Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 11: 791-796, 2005.*

Combating Toxic Iron Overload

Most people know that iron is essential for good health. What people may be surprised to learn is that too much iron—a condition called iron overload—can actually threaten health by damaging tissues and organs. Iron overload occurs in diseases such as hemochromatosis and Cooley's anemia. Although these conditions have different causes, they both involve the accumulation of excess iron, which is stored in the heart and/or liver. This excess iron can damage these organs so that they no longer work properly. Unfortunately, the human body does not have a natural way to rid itself of excess iron. To address these problems, NIH-funded research is providing insights regarding the regulation of iron metabolism and methods for removing toxic excess iron.

In hemochromatosis, genetic mutations alter control mechanisms that would otherwise precisely regulate iron absorption. The standard therapy for treating this condition is blood-letting (phlebotomy), which is equivalent to blood donation and is a relatively simple means of restoring normal iron stores in the body. However, research into the genetic basis of the disease could provide a platform for developing more effective treatments and prevention strategies. For example, a genetic screen to identify those at risk for hemochromatosis could permit early intervention (by phlebotomy) to prevent progression to organ toxicity caused by iron overload.

A decade ago very little was known about the genes that harbor the disease-causing mutations in hemochromatosis and the proteins these genes encode. A major advance occurred when mutations in a gene called *HFE* were discovered to underlie the most common form of the disease in humans.

The discovery of variants of the *HFE* gene that lead to mutant proteins provided an opportunity for early and rapid genetic identification of individuals at risk for development of hereditary hemochromatosis. A majority of patients diagnosed with iron overload due to hereditary hemochromatosis have been found to have two copies of a mutant *HFE* gene referred to as C282Y (one mutant gene copy inherited from each parent); a second *HFE* mutation (H63D) has also been associated with hemochromatosis but only if it occurs in association with C282Y. To further explore the potential for population screening for common *HFE* mutations, NIDDK-supported researchers screened a large number of people for the presence of *HFE* mutations and associated symptoms of hemochromatosis. In the study, among the individuals found to have two copies of the C282Y mutation, many had elevated iron levels. Researchers also observed an increase in liver disorders seen in those with *HFE* mutations, but did not observe greater frequency of most other symptoms characteristically associated with hemochromatosis. In fact, most of the people with mutant *HFE* genes had not developed clinical symptoms and signs of organ toxicity, indicating that having the *HFE* mutations does not guarantee clinical symptoms. This study revealed that additional mutations or environmental factors contribute to hereditary hemochromatosis. In a more recent study of an even larger population, NIH-funded researchers found similar results. Their study also revealed that the C282Y mutation likely does not account for high iron levels seen in non-Caucasian populations; the C282Y mutation had previously been found to be more common in Caucasians than in other groups. These findings will likely spur new research to identify other genetic or environmental factors that influence the development of clinical symptoms of hemochromatosis.

STORY OF DISCOVERY

Other insights into hemochromatosis emerged from studies showing the significance of hepcidin as a regulatory protein that controls iron balance in the body. These findings emerged from a series of seemingly unrelated studies in mice. One group of researchers found that mice fed a diet high in iron express in their livers high levels of *Hepc*, a gene encoding the antimicrobial peptide hepcidin. Other scientists made the fortuitous finding that iron overload develops in mice carrying a genetic mutation (in the upstream stimulatory factor 2 gene, *Usf2*). Additional experiments indicated that these mutant mice have completely lost their ability to express the *Hepc* gene in the liver. A year later, this same group of scientists reported that forced overexpression of the *Hepc* gene in the liver of mice led to offspring with pale skin, anemia, and decreased body iron levels—with death frequently occurring within a few hours of birth. These mice thus displayed characteristics of an experimental form of iron deficiency. Collectively, these studies highlight the important role the liver plays in sensing excessive levels of iron.

But how does hepcidin—the protein product of the *Hepc* gene—regulate the level of iron that is transferred from the intestine into the circulation? NIH-supported scientists hypothesized and demonstrated that, by binding to and destroying an iron exporter (FPN1), hepcidin prevents the transfer of iron from the intestinal cell into the circulation. Hepcidin causes the iron to be retained in the intestine cell, and thus protects other tissues and organs from iron overload. In addition, it is now known that hemochromatosis patients carrying mutations in several different genes each have inappropriately low levels of hepcidin.

Another group of patients who face problems with iron overload are those affected with anemias, such as Cooley's anemia, which require lifelong blood transfusions as a treatment regimen. Transfusions provide anemia patients with desperately needed functional red blood cells that their bodies cannot make in sufficient quantity. However, there is a serious downside to this treatment. Because the transfusions provide iron-rich blood directly into the circulation, they by-pass the normal intestinal control of iron absorption. As a result, toxic levels of iron build up in blood and body tissues—producing symptoms similar to those of untreated hemochromatosis. To combat iron overload, the NIH has supported research to develop agents, known as iron chelators, to remove excess iron from the body. The standard chelator, deferoxamine, generally must be administered under the skin using a pump for 10 to 12 hours per day, 5 days per week. Although this intervention will extend life in chronic severe anemias, it is enormously time-consuming, inconvenient and painful for patients, especially young patients, who find compliance with this regimen extremely difficult and frustrating. A new oral chelator has recently come on the market, but it alone may not remove sufficient iron to prevent lifelong problems.

To improve treatment options for patients with chronic severe anemia, the NIH continues to seek new and better iron chelators. In animal tests, one new agent (HBED) has been shown to remove three times more iron than the standard chelator. Although this new chelator must be injected, researchers hope that treatment may be limited to one short injection two or three times a week, which would be less demanding on patients. Phase I clinical trials of this new chelator are under way, supported by the pharmaceutical industry. The NIH is also supporting the adaptation of magnetic resonance technology for the measurement of body iron. This non-invasive technique would be enormously helpful in monitoring the effectiveness of new iron chelating agents and in the possible refinement of therapeutic approaches aimed at achieving iron homeostasis in patients.

Multiple studies by many research teams have shed new light on the mechanisms by which iron is regulated with respect to absorption and movement throughout the body. These research findings are thus helping to lay the groundwork for more accurate means of screening for genetic hemochromatosis and better and safer treatment strategies for combating iron overload in patients with hemochromatosis or Cooley's anemia. The NIH will continue to propel the translation of these and other discoveries into improvements in patient care.

PATIENT PROFILE

Joe Crossan

Hemochromatosis Means Excess Iron Build-up in the Body, Which Can Spell Trouble

Nearing 58 years of age, Joe Crossan thought the only health concerns he needed to deal with were elevated cholesterol levels, somewhat higher than normal blood pressure and being overweight by about 20 pounds. He had no idea that his body was insidiously harboring excess amounts of iron that, if left untreated, could eventually lead to a wide range of serious health conditions, including liver disease, heart abnormalities, damage to his pancreas and adrenal gland, arthritis, thyroid deficiency, and more.

"I was being treated for my cholesterol and having my blood tested when the results came back and I was told that my iron and red blood cell counts were both extremely high." His physician immediately referred him to a hematologist/oncologist specialist…and Joe began thinking that something was seriously wrong.

After conducting a battery of tests, the specialist told Joe that he had some bad news and some good news. The bad news was that Joe suffers from hemochromatosis, an inherited disorder that causes the body to absorb and store too much iron. The extra iron builds up in organs and damages them. Without treatment, the disease can cause these organs to eventually fail. The good news is that treatment for hemochromatosis is simple, inexpensive, and safe.

Just as Joe was able to control his cholesterol, blood pressure and weight through diet and exercise, he was able to get his iron and red blood cell counts well into the normal range through phlebotomies—

Joe Crossan

therapeutic blood-lettings to remove the excess iron from his circulation. In addition, Joe volunteered to participate in an NIH study to determine what damage, if any, his hemochromatosis may have already done to his heart or other organs. He underwent a variety of tests, including magnetic resonance imaging (MRI) to detect iron deposits in vital organs, such as the liver and pancreas; echocardiograms to visualize the heart and assess its function; a video scan of his liver, pancreas and thyroid; a bone density test; and more. Except for some thickening of the wall in one of the four chambers of his heart, "everything else proved negative," says Joe.

Joe was lucky. The disease had been discovered before the iron build-up had time to seriously damage any of his organs. However, because hemochromatosis is usually hereditary, Joe believes his father

PATIENT PROFILE

and mother may not have been as fortunate. His father died of a heart attack at age 62. His mother, though she lived to be 88, experienced severe arthritic pain (a symptom of the disease) to the point that, in her later years, she was almost completely disabled. "I believe both my parents may have suffered from hemochromatosis that had never been diagnosed," says Joe.

Joe, on the other hand, is now 60 years old, retired, and looking forward to continuing his lifelong passions of fishing and woodworking, and to spending time with his wife and four grown children. "I feel extremely fortunate," he says.

About Hemochromatosis

Genetic or hereditary hemochromatosis is one of the most common genetic disorders in the United States and is primarily associated with a defect in a gene called *HFE*. There are two known, important genetic mutations in *HFE*, termed C282Y and H63D. C282Y is the most important. When C282Y is inherited from both parents, iron is overabsorbed from the diet and hemochromatosis can result. Someone who inherits the mutated gene from only one parent is a carrier for the disease, but usually does not develop it.

Interestingly, recent studies have revealed that not everyone who inherits two copies of C282Y develops clinical symptoms of hemochromatosis. Researchers are still studying why this is so. One hypothesis is that variations in other genes involved in iron absorption and metabolism "modify" the disease manifestation (phenotype) of hereditary hemochromatosis. (See the accompanying "Story of Discovery: Combating Toxic Iron Overload.")

As a middle-aged, white male of Irish/German decent, Joe presented the stereotypical profile for the disease. The genetic defect of hemochromatosis is present at birth, but symptoms rarely appear before adulthood. Also, the disease is less common in African Americans, Asian Americans, Hispanic Americans, and American Indians. And although men and women can inherit the gene defect, men are about five times more likely to be diagnosed with the effects of hereditary hemochromatosis than women.[1]

Excess iron in the body, if not detected early and treated, may eventually lead to serious health problems, such as:

- Arthritis
- Liver disease, including an enlarged liver, cirrhosis, cancer and liver failure
- Damage to the pancreas, possibly causing diabetes
- Heart abnormalities, such as irregular heart rhythms or congestive heart failure
- Impotence
- Early menopause
- Abnormal pigmentation of the skin, making it look gray or bronze
- Thyroid deficiency
- Damage to the adrenal gland

Joint pain is the most common complaint of people with hemochromatosis. Joe, in fact, experienced some minor pain in his knuckles, but because his mother suffered with arthritis, he didn't think much of it. He also had some skin discoloration on his fingers and the lower part of his legs.

Other common symptoms include fatigue, abdominal pain, and loss of sex drive. Symptoms tend to occur in men between the ages of 30 and 50, and in women over age 50. Many people, however, may have no symptoms when they are diagnosed. In fact, because it is considered a rare disorder, and initial symptoms often mimic the symptoms of many other diseases, hemochromatosis often goes undiagnosed. Also, physicians may focus on the conditions caused by the disease—arthritis, liver disease, heart disease, and diabetes—rather than on the underlying iron overload itself.

Treating the Disease

Once diagnosed, the first step in treating hemochromatosis is to rid the body of excess iron. The process is called phlebotomy, which simply means removing blood—which, in turn, removes excess iron from the circulation, allowing total body iron to gradually drop back to normal levels. The blood is drawn the same way it is drawn from donors at blood banks, which means "the treatment is no more painful than giving blood," confirms Joe. Depending on the severity of the iron overload, a pint of blood is taken once or twice a week for several months to a year, occasionally longer, and then on a more intermittent schedule.

When Joe was first diagnosed with the disease, his level of ferritin—a measure for the concentration of iron in the blood, expressed as nanograms per milliliter of serum—was 1650; normal levels are between 12 and 300.[2] It took 35 withdrawals and nearly a year before his ferritin levels returned to within the normal range. During the treatment period, Joe felt fatigued. As his ferritin numbers decreased, the skin discoloration in his fingers and lower legs began to recede, as did the pain in his joints. "They weren't stiff anymore," says Joe. He is currently in the maintenance stage of his treatment, in which blood is withdrawn every few months, instead of nearly every week.

At one point during the treatment, Joe asked whether the blood that was being drawn from him was good. He was told that his blood was "very good," perhaps too rich in iron for someone like Joe, but fine for someone with anemia, who could benefit from healthy, iron rich blood. When Joe learned that his blood was being discarded, he told the physician, "I'd like to do something else with it." Joe's blood is now being infused into a person with anemia caused by sickle cell disease, and Joe is extremely pleased to know that his blood is being put to good use. "It's very satisfying to know that I'm helping someone else with his or her health crisis," says Joe.

Although treating hemochromatosis is relatively easy, there is no simple, inexpensive, and accurate test for routine screening, and the options that do exist have their limitations. DNA testing, for example, provides a definitive diagnosis, but is expensive, and currently cannot be used to accurately predict who will develop disease symptoms. Blood tests for transferrin saturation are widely available and relatively inexpensive, but unless performed carefully and more than once, may not produce a correct diagnosis.

Therefore, health professionals recommend the following:

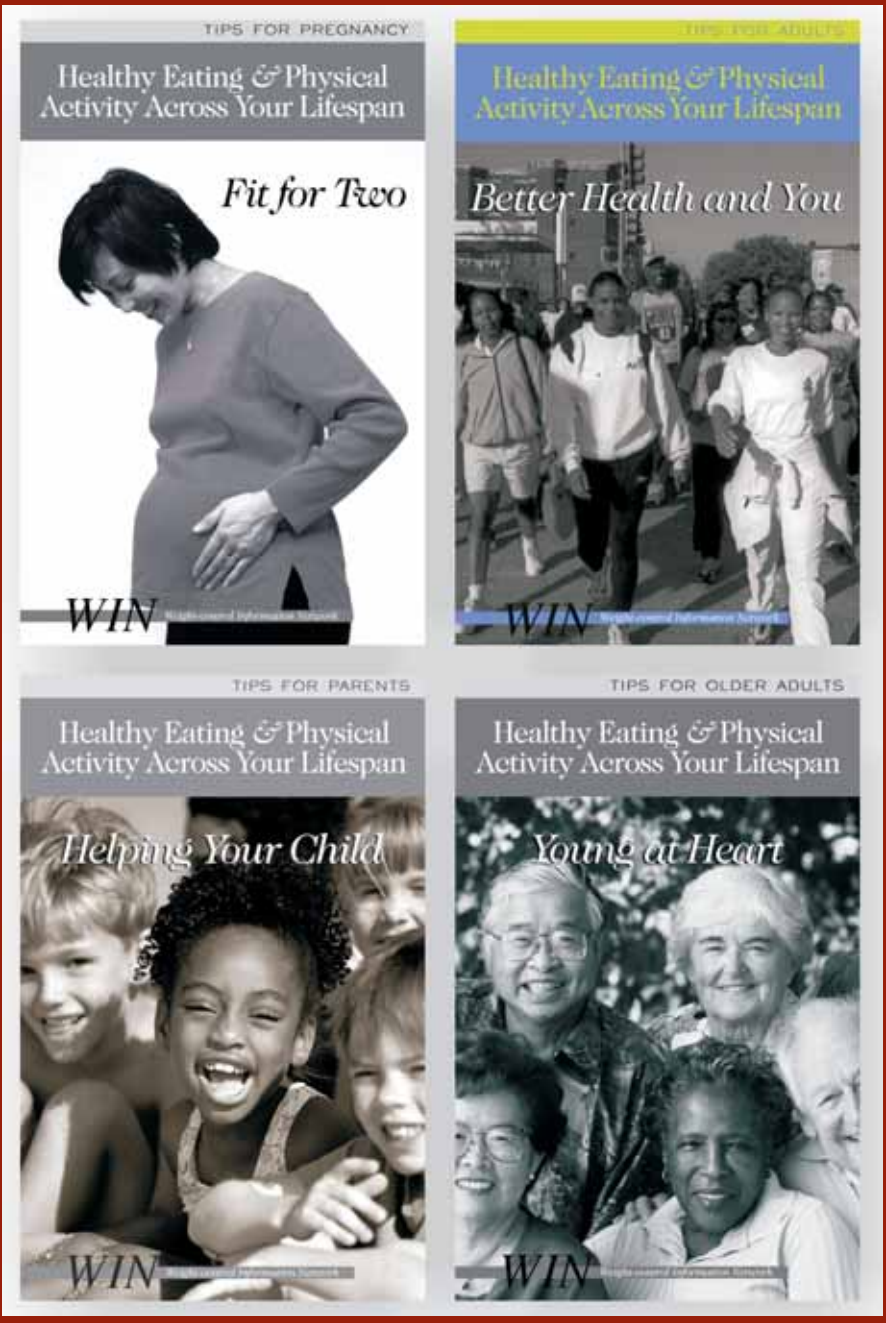
- Brothers and sisters of people who have hemochromatosis should have their blood tested to see if they have the disease or are carriers.
- Parents, children and other close relatives of people who have been diagnosed with the disease should consider being tested.
- Doctors should consider testing people who have joint disease, severe and continuing fatigue, heart disease, elevated liver enzymes, impotence, and/or diabetes, because these conditions may result from hemochromatosis.

Joe's sister, for example, has the disease. As a result, he is urging others in his family to get tested. "Thirty years ago, when my father died, very little was known about hemochromatosis. Thanks to research, a lot is known today about the disease. People at risk, at the very least, need to have their blood tested, and if their ferritin levels are found to be high, they need to be treated."

---

[1] *http://digestive.niddk.nih.gov/ddiseases/pubs/hemochromatosis/*

[2] *http://www.nlm.nih.gov/medlineplus/ency/article/003490.htm*



The NIDDK's Weight-control Information Network (WIN) provides the general public and health professionals with up-to-date, science-based information on obesity, weight control, physical activity, and related nutritional issues. WIN's series of publications, entitled "Healthy Eating and Physical Activity Across Your Lifespan," is available in English and Spanish and addresses people of all ages.

Obesity

Obesity has risen to epidemic levels in the U.S. Obese individuals suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. A strong risk factor for type 2 diabetes, obesity is also associated with other health conditions within the NIDDK's mission, including, for example, urinary incontinence, gallbladder disease, and the fatty liver disease nonalcoholic steatohepatitis.

Nearly 31 percent of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height. Furthermore, while obesity and overweight have risen in the population in general, the greatest increases observed over approximately the past two decades have been in the prevalence of extreme obesity; those who are severely obese are most at risk for serious health problems. Levels of childhood overweight have also escalated in the past several decades; approximately 16 percent of children and teens ages 6 through 19 are now overweight. (This document uses the terms overweight and obesity interchangeably for children and adolescents because there is no generally accepted definition for obesity, as distinct from overweight, in this age group.) The levels of pediatric overweight have ominous implications for the development of serious diseases both during youth and later in adulthood. Overweight and obesity also disproportionately affect racial and ethnic minority populations, and those of lower socioeconomic status.

The increased prevalence of obesity in the U.S. is thought to result from the interaction of genetic susceptibility with behavior and factors in the environment that promote increased caloric intake and sedentary lifestyles. Thus, the NIDDK has been supporting a multidimensional research portfolio on obesity ranging from basic studies to large clinical trials. This research includes, for example, investigations to elucidate the hormones and signaling pathways that influence appetite and energy expenditure; exploration of genetic factors that predispose individuals to obesity; studies of nutrition, including diet composition; research encompassing physical activity; and studies aimed toward obesity prevention through the development and testing of modifications of environmental factors in schools, the home, and other settings. The NIDDK additionally supports research on eating disorders that are associated with obesity in some people.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. To help bring the results of research to the public and health care providers, the NIDDK also sponsors education and information programs. Given the importance of the obesity epidemic as a public health problem, and its relevance to the mission of the NIDDK, the Institute has played a leading role in the NIH Obesity Research Task Force. Established by the NIH Director and co-chaired by the Directors of the NIDDK and the National Heart, Lung, and Blood Institute, the Task Force also includes representatives from numerous other NIH Institutes, Centers, and Offices. A major effort of the Task Force has been the development, with extensive input from external scientists and the public, of the *Strategic Plan for NIH Obesity Research*, published in August 2004 (*http://obesityresearch.nih.gov/About/strategic-plan.htm*).

ROLE OF GENETICS IN OBESITY

Identification of Genetic Mutations Associated with Obesity and Risk for Type 2 Diabetes in Children and Adults: The complexity of the genetics of obesity and type 2 diabetes poses significant challenges to the identification of genes associated with these conditions, as variations in many genes likely contribute to susceptibility. Recently, a group of researchers used a genetic approach to identify a specific region of a human chromosome as the likely location of a gene associated with childhood obesity (chromosomes are the large genetic structures that contain genes). The researchers subsequently pinpointed mutations in a gene within this region as associated with both obesity and type 2 diabetes in children and adults. This gene, called *ENPP1*, was

previously known to encode a protein that disrupts signaling by the hormone insulin; it does this by inhibiting actions of a critical cellular partner for insulin, the insulin receptor. Importantly, reduced responsiveness to insulin signaling (insulin resistance) can lead to type 2 diabetes. Further experimental analysis indicated that *ENPP1* protein levels are higher in children with obesity-associated mutations in the *ENPP1* gene. The scientists also found that one form of the *ENPP1* gene is "turned on" specifically in cells with particular relevance to obesity and diabetes: fat cells, pancreatic beta cells (which produce insulin), and liver cells. This study suggests a genetic mechanism for the link between childhood obesity and the high risk of type 2 diabetes in adolescence and early adulthood, and may help in the search for additional predisposing genes. Furthermore, these findings present new opportunities for strategies to prevent and treat obesity and diabetes.

Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, Ghoussaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, and Froguel P: Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet 37: 863-867, 2005.

LINKS BETWEEN OBESITY AND RISK OF TYPE 2 DIABETES

Obesity and Insulin Resistance Linked by a Circulating Protein: Researchers have identified a circulating protein that seems to contribute to insulin resistance in obese people and those with type 2 diabetes. Retinol binding protein 4, RBP4, is a protein secreted by fat cells, and its suppression may have therapeutic benefits. The researchers found that engineering mice to lack RBP4 showed improved insulin sensitivity, whereas inducing high levels of RBP4 protein expression, or administering purified RBP4 protein, resulted in insulin resistance. The putative negative effects of elevated RBP4 protein levels in mice have been reinforced by the observation that RBP4 is also elevated in humans with obesity and type 2 diabetes. Thus, RBP4 is a factor that may play a causative role in the development of insulin resistance and type 2 diabetes, and it may be a valuable clinical target for new drug therapies. Laboratory studies have already shown that a drug that promotes excretion of RBP4 in urine can improve insulin sensitivity in obese mice. With further investigations, it may be possible to translate these promising results of basic research into clinically-oriented studies that may benefit obese, insulin-resistant individuals who are prone to or affected by type 2 diabetes.

Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, and Kahn BB: Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436: 356-362, 2005.

FIDGETING AND BODY WEIGHT

Possible Role for Non-Exercise Activity in Preventing Obesity: Scientists are gaining insights into factors that influence weight gain—a problem that is on the rise in America. Today approximately 64 percent of U.S. adults age 20 and over are overweight or obese, with nearly 31 percent meeting criteria for obesity. Weight gain occurs whenever a person's energy intake exceeds his or her energy expenditure; however, it is challenging to tease out what distinguishes people who gain weight from those who do not. It is surprisingly difficult to precisely track every calorie a person consumes and every calorie he or she expends. Recent work has taken a major step in addressing the latter problem with a novel approach to assessing activity level. Researchers recruited 20 volunteers, 10 of whom were lean and 10 of whom were mildly obese, but all of whom were self-described "couch potatoes." For two weeks, the volunteers, who were wearing sensors that monitored their activity, followed their usual day-to-day activities and did not adopt any new exercise routines. During this time, the scientists collected data twice per second to determine whether the volunteers were sitting, standing, or lying down. They found that the lean subjects stood and/or moved, on average, two hours longer per day than those in the obese group. This type of energy expenditure is called "non-exercise activity thermogenesis," or NEAT. Thus, an important factor in maintaining leanness appears to be the expenditure of energy through movements that are not a component of intentional exercise.

Levine J, Lanningham-Foster L, McCrady S, Krizan A, Olson L, Kane P, Jensen M, Clark M: Interindividual variation in posture allocation: possible role in human obesity. Science 307: 584-586, 2005.

Lifestyle Modification Plus Medication Proves More Effective than Medication Alone for Weight Loss in Obese Adults

People who are obese are at risk for serious health problems, such as type 2 diabetes and cardiovascular disease, yet losing weight and maintaining weight loss are difficult to achieve. A new study shows that treatment with a lifestyle modification program of diet, exercise and behavioral therapy, when used in combination with the weight-loss medication sibutramine, resulted in significantly greater weight loss among obese adults than treatment with the medication alone.

A total of 224 obese adults participated in the one-year study. Participants were randomly assigned to one of four groups: 1) weight-loss medication alone; 2) lifestyle modification alone; 3) weight-loss medication plus lifestyle modification; and 4) weight-loss medication plus brief physician-mediated therapy. All groups were prescribed a 1,200 to 1,500 calorie diet and the same exercise plan. Participants in the group receiving weight-loss medication therapy alone met with primary care physicians eight times for 10 to 15 minute visits, but were not instructed to keep food or activity records and were provided only general information on diet and exercise. Participants assigned to the lifestyle modification therapy attended a total of 30 group meetings, each lasting 90 minutes. During the meetings participants were instructed to complete and share weekly assignments, which included keeping detailed daily food and physical activity records. Those participants in the combined therapy group received both the lifestyle modification therapy and the weight-loss medication. Participants in the medication plus brief physician-mediated therapy group met with primary care physicians eight times for 10 to 15 minute visits during which they were also given homework assignments which included keeping daily food and activity records.

After one year, patients in the weight-loss medication plus lifestyle modification (combined therapy) group, lost an average of more than 26 pounds—more than double the weight loss seen with medication alone (11 pounds). Interestingly, those participants in the combined therapy group who were most successful were those who frequently recorded their food intake. Those participants with high adherence to food-intake record keeping lost more than twice as much weight as those with low adherence (41.5 *versus* 17 pounds).

One limitation of the study is that it only included obese patients who were otherwise healthy and excluded obese patients with health problems possibly related to their obesity, such as hypertension, cardiovascular disease, cerebrovascular disease, kidney disease, liver disease, and diabetes. Because many obese patients also have other conditions that can adversely affect their health, physicians should carefully monitor patients enrolled in weight-loss programs that include weight-loss medications.

The findings of the study are consistent with the NIH's *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults*. These guidelines, developed by the National Heart, Lung, and Blood Institute in collaboration with the NIDDK, recommend that weight-loss medications be used in a supportive role to a comprehensive program of behavioral treatment, diet therapy, and increased physical activity. The guidelines also recommend that physicians prescribe a regimen of lifestyle therapy for at least six months before adding weight-loss medication to the regimen. That is, lifestyle modification should be the first line of treatment for obesity, but for obese adults who cannot lose enough weight to improve their health, medication used as an adjunct can help. A recent editorial in the *New England Journal of Medicine* underscores not only the need for improved medical approaches to treating obesity but also the potential dangers and limitations of weight-loss drugs. Weight-loss medication will be most effective when combined with a reduced-calorie diet and increased physical activity.

Wadden TA, Berkowitz RI, Womble LG, Sarwer DB, Phelan S, Cato RK, Hesson LA, Osei SY, Kaplan R, and Stunkard AJ: Randomized trial of lifestyle modification and pharmacotherapy for obesity. N Engl J Med 353: 2111-20, 2005.

Yanovski SZ: Pharmacotherapy for obesity—promise and uncertainty. N Engl J Med 353: 2187-2189, 2005.

NIDDK's Weight-control Information Network (WIN)

The NIDDK's Weight-control Information Network (WIN) provides the general public and health professionals with up-to-date, science-based information on obesity, weight control, physical activity, and related nutritional issues. This information includes fact sheets and brochures for the public, as well as WIN Notes, a periodic newsletter for health professionals and consumers. Through its information services, WIN reaches out to people of all ages, and to diverse ethnic and racial groups.

In addition to developing science-based fact sheets and brochures, WIN conducts a variety of ongoing outreach activities to primary care providers, registered dietitians, fitness professionals, health educators, community organizers and others. Recent promotional efforts include a focus on the benefits of regular physical activity, tips to help overweight children, weight loss and nutrition myths, and portion control.

To promote the availability of its brochure series, "Cómo Alimentarse y Mantenerse Activo Durante Toda La Vida" ("Healthy Eating and Physical Activity Across Your Life Span"), WIN recently conducted an outreach effort in U.S. cities with a high proportion of low-income Latino residents. The cities were Los Angeles, New York, Miami, and Washington, D.C. The "Toda La Vida" series of booklets, available in English and Spanish, provide Spanish-speaking adults, older adults, parents, and pregnant women with tips on how to build regular physical activity and healthful eating into these four stages of life and beyond.

WIN's "Sisters Together: Move More, Eat Better," is a national initiative that encourages African American women to maintain a healthy weight by becoming more physically active and eating more healthful foods. Among its publications are: "Celebrate the Beauty of Youth!," "Fit and Fabulous as You Mature," "Energize Yourself and Your Family," and "Walking...A Step in the Right Direction." WIN conducts ongoing outreach efforts to historically Black colleges and universities and various community venues to promote the availability of "Sisters Together" brochures.

Fat Metabolism and Obesity

Dr. Samuel Klein

Dr. Samuel Klein received his M.D. from Temple University in 1979, and an M.S. in Nutritional Biochemistry and Metabolism from MIT in 1984. He completed residency and a clinical nutrition fellowship at University Hospital in Boston, as well as fellowships at Harvard Medical School in Boston and Mt. Sinai Hospital in New York. He is currently the William H. Davenport Professor of Medicine and Nutritional Science at the Washington University School of Medicine in St. Louis, where he also serves as Director of the Washington University Clinical Nutrition Research Unit, Director of the Weight Management Center, and Associate Program Director of the General Clinical Research Center. He discussed the work from his laboratory at the February 2005 meeting of the NIDDK's Advisory Council. Some highlights of his talk are presented here in this "Scientific Presentation."

The ability of the human body to store excess energy as body fat has proved invaluable during human history for overcoming periods of famine. However, in the U.S. today, food is relatively plentiful, high-calorie food is comparatively inexpensive, and technologic advances have led many individuals into sedentary lifestyles. These and other factors have contributed to an upsurge in rates of overweight and obesity. Because excess body fat is associated with numerous serious diseases—including type 2 diabetes, heart disease, nonalcoholic fatty liver disease and many others—the health consequences of this trend toward increasing weight are severe. Through studies of fat metabolism, the Klein lab seeks to understand the metabolic effects of different weight-loss treatment approaches and the link between excess fat and the metabolic abnormalities seen in diseases associated with overweight and obesity.

Obesity is frequently associated with serious medical complications, as illustrated in this figure. Among these complications are higher than normal levels of unhealthy blood fats, and/or lower than normal levels of healthy blood fats ("dyslipidemia"); inflammation of blood veins ("phlebitis"); and joint pain caused by the accumulation of uric acid deposits ("gout"). Cancers more common in people with obesity include those of the breast, uterus, cervix, esophagus, pancreas, liver, kidney and prostate. Gynecological conditions sometimes associated with obesity include abnormal menstruation, infertility and polycystic ovarian syndrome; pulmonary (lung) problems include abnormal function and obstructed breathing during sleep (apnea).

Image courtesy of Dr. Samuel Klein.

Insights into the Regulation of Fat Metabolism

The body stores fat in molecules called triglycerides. When fat is burned for fuel, each triglyceride is broken into its component parts: three free fatty acid (FFA) molecules and one molecule of glycerol. The Klein lab studies fat metabolism by directly measuring the appearance

and disappearance of the FFA and glycerol in the body when fat is utilized for energy. The presence of excess FFA is known to stimulate the liver to release glucose and to contribute to type 2 diabetes by inhibiting the action of insulin. Excess FFA also stimulates the production of very low density lipoprotein (VLDL, which is the primary carrier of plasma triglycerides), and decreases high density lipoprotein-cholesterol (HDL-cholesterol, also known as "good cholesterol"). Both of these effects increase the risk of heart disease.

Dr. Klein and his colleagues measured fat metabolism (breakdown of fat into glycerol and FFA) in lean women and in obese women, and found that the latter have lower rates of FFA and glycerol release than their lean counterparts, when measured per gram of fat. However, because the obese women had so much more fat than the lean women, they had a much higher total amount of FFA and glycerol release. Thus, because excessive FFA is so unhealthy, the obese body may partially compensate by reducing the rate of fat metabolism—but cannot reduce it sufficiently to keep FFA at a healthy level.

No Effect of Liposuction on Fat Metabolism

Losing even a modest amount of weight, however, can improve an overweight person's metabolic profile very quickly. Indeed, there is a dramatic drop in the release rate of FFA associated with weight loss. There are also major improvements in blood glucose and insulin action. However, numerous studies have demonstrated that it is very difficult for people who lose weight to keep that weight off. So the Klein team investigated whether they could achieve the metabolic benefit of weight loss by simply removing fat from obese people using the cosmetic surgery known as liposuction.

They found that liposuction had no effect on metabolic risk factors, even when very large quantities of fat were removed. One possible explanation for this comes from the fact that liposuction only removes subcutaneous fat (fat in the layer just beneath the skin). It may be that the fat depots in and around organs such as liver or in muscle have adverse effects and cause insulin resistance, and other risk factors for heart disease seen in clinical obesity. Another possibility is that—when weight loss occurs because calories expended exceed calories consumed (as in successful diet and exercise programs)—fat cells shrink in size. In liposuction fat cells are removed, but the remaining cells do not become smaller. It is possible that the size of fat cells is a significant factor in regulating fat metabolism. This question will require further investigation.

Effects of Diet and Exercise on Fat Metabolism

Dr. Klein reviewed the work by his group and others that compares low-fat diets with low-carbohydrate diets, or with calorie-restricted diets. He noted that there was a remarkably consistent finding across investigators that little difference occurred in overall weight loss after a year or more on these diets, even though people on low-carbohydrate diets tend to lose more weight in the short term. However, he pointed out that the low-carbohydrate dieters do fare better with respect to several markers of cardiovascular disease, particularly their levels of triglycerides and HDL-cholesterol, which improve more in low-carbohydrate dieters than in low-fat dieters. More research will be necessary to establish the reasons for this difference, and whether it actually translates to improved health outcomes.

Of course, an exercise program is also considered to be a critical part of most weight-loss efforts. Dr. Klein noted that, in principle, it is simpler to avoid consumption of a high calorie item (or to substitute a "diet" version) than to burn the equivalent number of calories via exercise. Nonetheless, exercise is associated with successful maintenance of weight loss. Importantly, it is not clear whether successful weight loss is the result of dieting, or whether individuals who can maintain an exercise program are also particularly likely to adhere consistently to their diets.

Dr. Klein noted that exercise can be a powerful stimulus for the burning of fat as fuel. Examining the

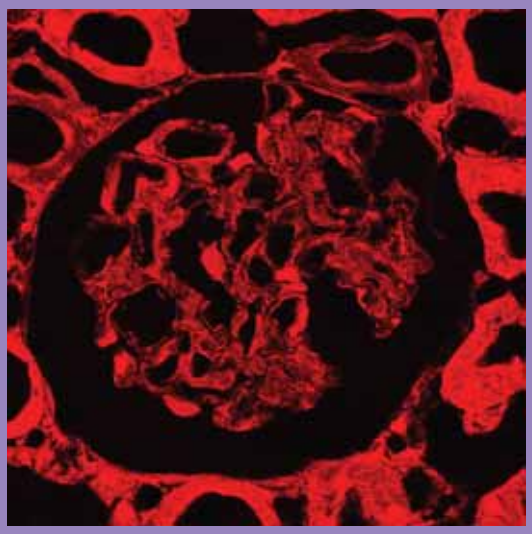
body's ability to use fat for fuel during endurance exercise, the Klein lab noted that study volunteers used more fat during a particular session of exercise after 16 weeks of training than before training began. This means that the body is adapting in some fashion to the exercise program. The investigators discovered that the change occurred because muscle tissue becomes better adapted for consuming fat calories: specific proteins used in burning fat for fuel are increased after 16 weeks of training.

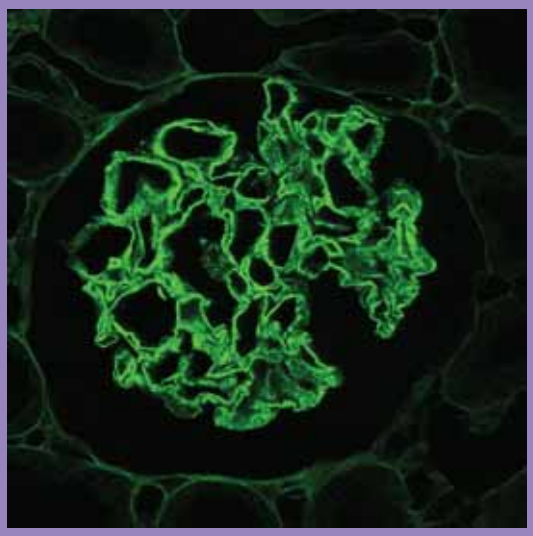
Effect of Gastric Bypass Surgery on Fat Metabolism and Fatty Liver Disease

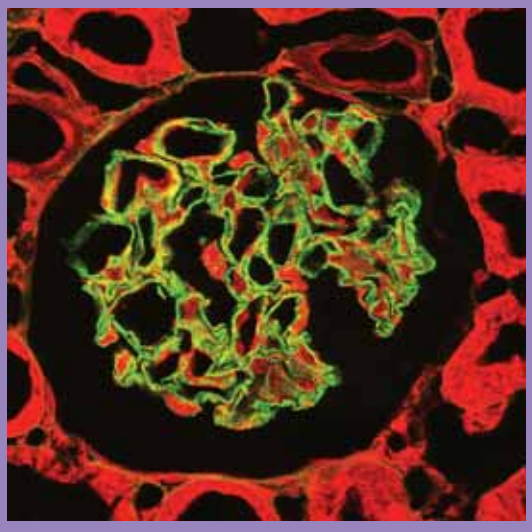
In the last portion of his presentation, Dr. Klein turned to an important complication of obesity—nonalcoholic fatty liver disease (NAFLD). NAFLD is caused by a chronic excess of fat in the liver (steatosis), an increasingly common problem that is associated with obesity. The Klein lab examined rates of fat metabolism in patients who underwent gastric bypass surgery as a treatment for obesity. Unlike liposuction, which directly removes fat, the gastric bypass procedure causes a marked decrease in caloric intake.

The Klein study focused on patients who had steatosis. The patients were found to have a significant drop in the rate of FFA release following their surgeries. The investigators also showed that liver VLDL-triglyceride secretion rate decreased because of a marked decrease in the contribution of FFA derived from intrahepatic or visceral fat breakdown to VLDL-triglyceride production.

Building up and extending these impressive findings, Dr. Klein and colleagues are next planning to analyze the molecular mechanisms governing these changes in fat metabolism, as well as the processes that lead from steatosis to nonalcoholic steatohepatitis. The Klein lab is actively studying these challenging questions, while also investigating the ominous links between obesity and other diseases. These studies, combining basic and clinical investigations, are a powerful tool for providing valuable insights into the complex metabolic regulation that affects body weight, makes maintaining weight loss so difficult, and contributes to the many diseases associated with obesity.







Using multiple antibodies linked to different fluorescent molecules, scientists can determine the location of various proteins with great specificity and high resolution. These three images show the pattern of expression of the protein TRPC6 in mouse kidney. The structure at the center of each image is one of the critically important filtering units of the kidneys, a glomerulus. Within these filtering units, TRPC6 is found primarily in a subpopulation of cells called podocytes. Red fluorescent antibodies (top) detect expression of TRPC6 in two types of cells in the glomerulus and in the surrounding tubules. Green fluorescent antibodies (middle) detect synaptopodin, a protein specific to podocytes. When the fluorescence of both antibodies is seen together (bottom), the combined fluorescent pattern is yellow, confirming that TRPC6 expression within the glomerulus is confined largely to podocytes.

Image courtesy of Dr. Martin R. Pollak and reprinted with permission from Reiser et al. Nat Genet 37: 739-744, 2005.

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 is committed to enhancing research to understand, treat, and prevent these diseases.

Normal, healthy kidneys process about 200 quarts of blood a day to filter out about two quarts of waste products and extra water, excreting them as urine. In people with chronic kidney disease, the function of these life-sustaining organs is impaired. Kidney disease may 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. Between 1990 and 2000, the number of people with kidney failure requiring dialysis or transplantation more than doubled, to 380,000. The leading cause of kidney disease is diabetes, with hypertension (high blood pressure) the second-leading cause. If unchecked, the recent increases in obesity and type 2 diabetes in the U.S. will have grave implications in several years, as more people begin to develop kidney complications of diabetes.

Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease. African Americans are four times more likely and American Indians are twice as likely to develop kidney failure as non-Hispanic whites. Hispanics have a significantly increased risk for kidney failure, as well.

The NIDDK supports a significant body of research aimed at increased understanding of the biology underlying chronic kidney disease. The 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. Areas of focus include diseases that collectively account for more than half of all cases of treated ESRD. Of special interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related glomerular diseases, including IgA nephropathy and the hemolytic uremic syndrome. The National Kidney Disease Education Program, which 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, represents a major educational outreach effort to patients and physicians.

Urologic diseases affect men and women of all ages, result in significant health care expenditures, and—if misdiagnosed or improperly treated—may lead to substantial disability and impaired quality of life. The NIDDK's urology research portfolio includes basic and clinical research on the normal and abnormal development, structure, and function of the genitourinary (GU) tract. The NIDDK also supports studies of a number of noncancerous urologic diseases, including benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, and congenital anomalies of the genitourinary tract.

Benign prostatic hyperplasia, or BPH, is a serious condition that is especially common among older men. Prostatitis—chronic inflammation of the prostate gland—is a painful condition that accounts for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genital and urinary systems. The NIDDK is committed to enhancing research to understand, treat, and prevent these common and troubling disorders.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). Interstitial cystitis (IC) is a debilitating, chronic, and painful bladder disease. The number of individuals suffering with IC is not known with certainty, but it has been estimated as many as 1 million Americans may have the disease, and of those, up to 90 percent are

women. Millions of Americans, most of them women, suffer from urinary incontinence. Kidney stones, a condition formally known as urolithiasis or urinary tract stone disease, is a frequent cause of visits to health care providers. One of the most common causes of kidney failure in children is vesicoureteral reflux. In fact, abnormalities of the genitourinary tract are among the most common birth defects.

To address these and other urologic problems, the NIDDK's urology research efforts support basic, applied, and clinical research in prostate function and prostate diseases; diseases and disorders of the bladder; male sexual dysfunction; urinary tract infections; urinary tract stone disease; and pediatric urology, including developmental biology of the urinary tract. A research emphasis of the urology program is the study of chronic inflammatory disorders of the lower urinary tract.

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, and thrombocytopenia. The Institute is also keenly interested in the basic biology and genetic regulation of stem cells, especially 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.

CHRONIC KIDNEY DISEASE AND HEART DISEASE

Cardiovascular Mortality Risk in People with Chronic Kidney Disease: Elderly people with chronic kidney disease have a substantial risk of dying from cardiovascular disease (CVD). The Cardiovascular Health Study, involving a group of elderly men and women with a high prevalence of chronic kidney disease, has underscored the importance of combating "traditional" risk factors among this population. These factors include high blood pressure, diabetes, obesity, smoking, and left ventricular hypertrophy (enlargement of the lower left chamber of the heart, usually caused by high blood pressure). "Novel" risk factors for CVD, including markers of inflammation and prothrombotic factors (molecules that promote blood clotting) were also examined. Examining six traditional risk factors and six novel risk factors for CVD, researchers found that, in patients with chronic kidney disease, traditional risk factors were associated with the largest increases in CVD death and that the increases associated with the novel factors were smaller and not statistically significant. These findings suggest that interventions that target traditional risk factors—blood pressure control, blood sugar control, smoking cessation, and increased physical activity—may have the greatest potential to reduce CVD mortality in this high-risk population.

Shlipak MG, Fried LF, Cushman M, Manolio TA, Peterson D, Stehman-Breen C, Bleyer A, Newman A, Siscovick D, and Psaty B: Cardiovascular mortality risk in chronic kidney disease: comparison of traditional and novel risk factors. JAMA *293: 1737-1745, 2005.*

MOLECULAR FACTORS UNDERLYING KIDNEY DISEASE

An Ion Channel Plays a Role in Kidney Disease: Focal segmental glomerulosclerosis (FSGS) damages the filtering units of the kidneys, thereby allowing protein and sometimes red blood cells to leak into the urine. Many patients with FSGS progress to end-stage renal disease. The ion channel encoded by the *TRPC6* gene is thought to be an important contributor to the kidney damage seen in this disease, but its role has been unclear. In one recent study, researchers studying a large family with hereditary kidney disease identified a mutation in the *TRPC6* gene, which results in a protein with altered subcellular distribution that is hypersensitive to stimulation. In a second study, researchers described in fine detail the subcellular localization of the normal protein encoded by the gene within the kidney filters and identified a number of important structural proteins with which the gene interacts. They then identified five families with hereditary kidney disease, and found each had a different mutation in this gene. When expressed in cultured cells, two of these five mutants resulted in increased ion flow across the cell membrane—suggesting that the mutant proteins may alter normal functions in the kidney filters. These

two advances identify a novel mechanism for the kidney damage seen in FSGS. The development of agents that target the mutated TRPC6 protein may be a useful strategy in the treatment of chronic kidney disease.

Reiser J, Polu KR, Moller CC, Kenlan P, Altintas MM, Wei C, Faul C, Herbert S, Villegas I, Avila-Casado C, McGee M, Sugimoto H, Brown D, Kalluri R, Mundel P, Smith PL, Clapham DE, and Pollak MR: TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. Nat Genet 37: 739-744, 2005.

Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL, Pericak-Vance MA, Howell DN, Vance JM, and Rosenberg PB: A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. Science 308: 1801-1804, 2005.

Signaling Pathways in Kidney Fibrosis: New research clues could lead to prevention strategies for a major cause of kidney damage—the scarring of kidney tissue (fibrosis). Insights are emerging about bone morphogenic proteins (BMPs), which not only induce bone formation, but also play an important role in embryonic development. One of these proteins, BMP-7, is key to the development of the kidney. Signaling by BMPs is mediated through cell surface receptors. Their activity is known to be inhibited by proteins, such as chordin and noggin, that prevent them from binding to their receptors. Scientists have now identified another BMP-binding protein, called KCP, which is similar in structure to chordin, but which enhances BMP-7 activity. Found in embryonic brain, limb buds, and kidney, this protein seems to have its enhancing effect by promoting the binding of BMP-7 to its receptor. Using two animal models of kidney damage, researchers found that mice lacking KCP were more susceptible to kidney damage. In one model, these mice also had a significantly higher death rate and a more problematic recovery compared to normal mice. Kidney fibrosis is a common clinical feature of many forms of chronic kidney disease, and it can contribute to irreversible kidney failure. Thus, enhancing BMP signaling with KCP-like agents may have important clinical implications.

Lin J, Patel SR, Cheng X, Cho EA, Levitan I, Ullenbruch M, Phan SH, Park JM, and Dressler GR: Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. Nat Med 11: 387-393, 2005.

COSTS OF KIDNEY STONES

Kidney Stones: A Growing Health and Economic Burden: Researchers are gathering data about the natural history and economic impacts of kidney stones, also known as urolithiasis, which are solid masses formed from minerals that are dissolved in urine. Kidney stones are an increasing problem in the U.S., and the recurrence rate of kidney stones has been estimated to be as high as 50 percent at 5 years. Treatment of kidney stones may require physician visits, hospitalizations or surgical interventions. Between 1994 and 2000, the number of hospitalizations and the average length of hospital stays for urolithiasis decreased 15 percent. However, due to the emergence of less invasive treatment options, the number of outpatient visits increased by 40 percent and physician office visits increased 43 percent between 1992 and 2000. Overall, kidney stone-related expenditures rose 50 percent from 1994 to 2000, with an annual total cost of $2.1 billion, despite a shift from costly inpatient procedures to less expensive outpatient procedures.

Pearle MS, Calhoun EA, and Curhan GC: Urologic diseases in America project: urolithiasis. J Urol 173: 848-857, 2005.

KEY EVENTS IN PROPER URINARY TRACT DEVELOPMENT

Insights into the Development of the Urinary Tract: Congenital malformations of the urinary tract are among the most common of all birth defects, and can cause renal failure and the need for dialysis or transplantation. Researchers have identified a previously unknown key event in urinary tract development that will aid in understanding the developmental process. Complete and efficient removal of toxic substances from the blood depends on tight connections among the kidneys, bladder, and interconnecting tubules called ureters. During embryonic development, one end of the ureter is attached to the nascent kidney and the other end is joined to the developing bladder through a structure called the common nephric duct (CND). The CND disappears during development. Previous models of ureter development posited that the CND underwent tissue remodeling and became a different structure. Scientists have now found that the CND is in fact lost during urinary tract development through a process of programmed cell death. The death of CND cells is dependent on signaling by vitamin A. Loss of the

CND is critical for the formation of the essential tight connection between the ureter and the bladder. This novel finding, which contradicts the previous model of ureter development, provides a new way to approach the biology and genetics of urogenital tract formation.

Batourina E, Tsai S, Lambert S, Sprenkle P, Viana R, Dutta S, Hensle T, Wang F, Niederreither K, McMahon AP, Carroll TJ, and Mendelsohn CL: Apoptosis induced by vitamin A signaling is crucial for connecting the ureters to the bladder. Nat Genet 37: 1082-1089, 2005.

UNDERSTANDING HEMATOLOGIC DISEASE

Insights into Hereditary Hemochromatosis: An important connection has been identified between two molecules involved in maintaining the delicately balanced metabolism of iron. Hemochromatosis is a disease in which abnormal iron metabolism results in the accumulation of toxic iron levels—termed iron overload—that eventually damages the liver, heart and other organs. Recent studies to combat this problem have focused on the hormone hepcidin, which is known to be a key player in the regulation of iron metabolism. Although deficiency in hepcidin has been implicated in some forms of hereditary hemochromatosis, the precise mechanism for hepcidin regulation of iron levels was not known. Scientists recently identified the protein ferroportin (Fpn), an iron exporter on the surface of some cells, as a receptor for hepcidin. In cell cultures, the binding of hepcidin to Fpn resulted in internalization and degradation of the complex, thereby preventing iron export by Fpn. Because Fpn exports iron absorbed by intestinal cells into the circulation, hepcidin-mediated destruction of Fpn may be key to regulating the dietary iron equilibrium. Researchers then studied several mutations in the Fpn gene that are linked to one type of hereditary hemochromatosis, and found that they either produced a protein that never arrives at the cell surface or one that does not internalize and degrade in the presence of hepcidin. Taken together, these findings suggest that loss of hepcidin regulation of Fpn levels—caused either by Fpn mutations or by deficiency in hepcidin—could explain, at least in part, the abnormal iron accumulation observed in hemochromatosis patients. A fuller understanding of the hepcidin-Fpn pathway in iron regulation will help to provide the foundation for future research aimed at treating or preventing iron overload disorders.

De Domenico I, Ward DM, Nemeth E, Vaughn MB, Musci G, Ganz T, and Kaplan J: The molecular basis of ferroportin-linked hemochromatosis. Proc Natl Acad Sci U S A 102: 8955-8960, 2005.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, and Kaplan J: Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306: 2090-2093, 2004.

U.S. Kidney Failure Rates Stabilize, Ending a 20-Year Climb, but Troubling Racial Disparities Persist

According to new data released by the NIDDK-supported United States Renal Data System (USRDS), rates for new cases of kidney failure have stabilized after 20 years of five to ten percent annual increases. Decreases have been noted each year for the last four years, and epidemiologists are now convinced these trends reflect consistent changes in the rate of disease. The good news is accompanied by bad news, however, since racial disparities in the rates of end-stage renal disease persist.

In 2003, the rate for new cases of kidney failure was 338 per million people, down slightly from 2002 and continuing a four-year trend. This has permitted researchers to be cautiously optimistic that rate decreases have not happened by chance. The average annual increase has been less than one percent since 1999, compared to five percent or more each year in the previous two decades.

Diabetes and high blood pressure remain the leading causes of kidney failure, accounting for 44 percent and 28 percent of all new cases, respectively. The most striking trends are seen in diabetes, where rates for new cases in Caucasians under age 40 were the lowest since the late 1980s, in stark contrast to rates for their African American counterparts, which have not changed.

The recent stabilization in kidney failure rates is likely attributable, at least in part, to better preventive care. The aging of the population and the increased numbers of diabetic patients are all trends tending to increase, not decrease, the number of people at risk of kidney disease. In the last two decades, however, clinical research, funded in part by NIDDK, has established the effectiveness of preventive strategies. The Diabetes Control and Complications Trial (DCCT) established the importance for patients with diabetes of good control of blood sugar and the value of monitoring for protein in the urine to detect early disease.

Other studies performed in the 1990s demonstrated that angiotensin-converting enzyme inhibitors (ACE-inhibitors) and angiotensin receptor blockers (ARBs) significantly delay or prevent kidney failure, particularly in patients with protein in the urine. Both types of drug decrease the amounts of protein in the urine. While ACE-inhibitors and ARBs are still underutilized, there has been a dramatic increase in their use. In the past decade, the use of these medications doubled among people over age 60 with chronic kidney disease, from 16 percent to 32 percent of patients, and they were also used by nearly half of those who also had diabetes or hypertension or congestive heart failure.

Still, despite incremental successes in preventing kidney failure and in improving health and survival of people who have it already, the increasing and aging U.S. population means that more people than ever before are living with the disease. The NIDDK has launched a program to increase awareness about kidney disease, the National Kidney Disease Education Program (NKDEP). The NKDEP encourages early diagnosis and management by increasing awareness about the connection between diabetes, high blood pressure and kidney disease; strategies proven to prevent or delay kidney failure; estimating kidney function to detect kidney disease earlier; and efforts to standardize testing for kidney disease and encourage more laboratories to automatically report estimated kidney function. Because of the higher rates of kidney disease seen in minority populations, the NKDEP has developed the "You Have The Power To Prevent Kidney Disease" campaign for African American adults and the *"¡Cuidado! La diabetes y la presión arterial alta pueden causar enfermedades de los riñones. Aprenda a proteger sus riñones"* ("Caution! Diabetes or High Blood Pressure Can Cause Kidney Disease! Learn how to protect your kidneys") campaign for Hispanics. Both programs are intended to increase awareness of kidney disease and the importance of

early detection in these minority populations who are disproportionally affected by the disease. "You Have The Power To Prevent Kidney Disease," was pilot-tested in 2003 in four cities—Atlanta, GA; Baltimore, MD; Cleveland, OH; and Jackson, MS—before being launched nationally in 2004. *"¡Cuidado! La diabetes y la presión arterial alta pueden causar enfermedades de los riñones. Aprenda a proteger sus riñones"* is a new NKDEP initiative, and was launched in January 2006. For more information about the NKDEP, see the accompanying sidebar, "National Kidney Disease Education Program (NKDEP)."

USRDS research depends on collaborations with other agencies of the U.S. Department of Health and Human Services (HHS), including the Centers for Medicare and Medicaid Services, the United Network for Organ Sharing, and the Centers for Disease Control and Prevention. Patient registries for other countries also contribute data for analyses.

Increasing Awareness of Interstitial Cystitis

Experiencing symptoms of pain around the bladder or pelvic area, and increased urge or frequency of urination, can disrupt normal life. Not knowing what is causing these symptoms—or what can be done about it—makes a difficult situation even harder. Interstitial cystitis (IC) is a painful and often debilitating bladder illness characterized by this syndrome of symptoms. Both IC and the related "painful bladder syndrome" (PBS) are currently diagnosed only by excluding other possible causes of these symptoms, such as infections or bladder cancer. Thus, IC is difficult to identify. Moreover, many patients and physicians are not familiar with these syndromes, and this lack of knowledge can delay diagnosis and possible treatment even further.

The NIDDK is helping to increase awareness of IC and PBS in order to help hasten patient diagnosis and access to information about treatment options. The Institute developed and launched an Interstitial Cystitis Awareness Campaign under the auspices of its National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC). Throughout 2005, the campaign targeted messages about IC to three audiences—the public, urologists, and general practitioners. For example, to increase awareness among urologists, the Clearinghouse developed and mailed an information package to members of the American Urological Association that included:

- A cover letter to AUA members from the NKUDIC
- Copies of the IC/Painful Bladder Syndrome fact sheet (available at *http://www.kidney.niddk.nih.gov/kudiseases/pubs/interstitialcystitis/index.htm*)
- A "Hope Through Research" fact sheet, "NIDDK: Solving the Puzzle of Interstitial Cystitis." This new publication was developed specifically for this mailing.

To reach primary care providers, the Clearinghouse provided information about IC at several broad-based professional meetings, including the annual conference of the American Academy of Family Physicians and the meeting of the American Academy of Physician Assistants. Finally, to increase awareness in the general public, the Clearinghouse developed and distributed a feature article about IC nationwide in 30 newspapers and weeklies, with a cumulative circulation of more than 500,000.

Many questions remain about which individuals develop IC and why, as well as how many people are affected in the U.S. and abroad. The NIDDK is supporting studies to obtain more precise answers to these questions, which will, in turn, assist in efforts to target information to patients and physicians. However, IC/PBS does appear to be far more common in women than in men. In the future, the IC Awareness campaign will become part of a new, multifaceted women's urologic health outreach program that is currently under development by the NIDDK.

The National Kidney Disease Education Program (NKDEP)

An estimated 20 million Americans currently suffer from chronic kidney disease (CKD), and millions more do not realize they are at risk. Treating the number of people with irreversible kidney failure, also called end-stage renal disease (ESRD), now costs the U.S. health care system more than $25 billion every year for dialysis and kidney transplantation. Although recent data from the NIDDK-supported USRDS indicate that ESRD rates are stabilizing after twenty years of annual five to ten percent increases, ESRD remains an enormous public health problem that disproportionately affects minority populations. Because the leading causes of kidney disease are diabetes and high blood pressure, the increasing prevalence of obesity and type 2 diabetes in the U.S. could fuel future rates of ESRD.

The NKDEP aims to raise awareness of the seriousness of kidney disease, the importance of testing those at high risk—those with diabetes, high blood pressure, cardiovascular disease, or a family history of kidney disease—and the availability of treatment to prevent or slow kidney failure.

The NKDEP emphasizes that effective treatments and management strategies for kidney disease exist, yet are being inadequately utilized. The progression from chronic kidney disease to kidney failure can be prevented or delayed if it is detected and treated early enough. Yet only a small number of the people who need proper screening or treatment receive it.

The NKDEP uses a multipronged approach to help achieve its goals. Toward this end, it is implementing public education and awareness initiatives; creating tools and programs for healthcare providers who play a key role in diagnosing and treating chronic kidney disease and its complications; and spearheading systemic change to improve the accuracy and automatic reporting of estimated glomerular filtration rate (GFR), a measure of kidney function.

Some current and recent activities of the NKDEP include:

Creatinine Standardization Program: The NKDEP's Laboratory Working Group is leading an effort to reduce bias in the measurement of serum creatinine, which is used to calculate GFR, and thereby estimate kidney function. GFR measures how well the kidneys are filtering waste, and is based on measurements of creatinine in the blood. Creatinine is a waste product, and healthy kidneys remove it from the blood and excrete it in urine. When the kidneys are not working well, creatinine builds up in the blood.

The standardization program encourages manufacturers of *in vitro* diagnostic equipment to recalibrate routine serum creatinine methods and to coordinate this recalibration with the introduction of a revised equation to estimate GFR.

Through information materials, the Laboratory Working Group also is encouraging laboratories to routinely report estimated GFR when serum creatinine is ordered. The NKDEP is in the process of developing a laboratory survey to gather baseline data that will be used to evaluate the success of this initiative.

Health Care Provider Outreach: NKDEP information is encouraging provider interactions with health systems, disease management companies, professional associations and others to encourage testing of at-risk patients and use of estimated GFR to increase early detection of kidney disease.

Communicating about Risk Factors—Family Reunion Initiative: The NKDEP conducted a pilot program to encourage African Americans to discuss the connection between diabetes, high blood pressure, and kidney disease at large family reunions. The "Kidney Connection Toolkit," the centerpiece of the initiative, provided kidney disease background information and guides to facilitate communication about risk factors for kidney disease and the steps people can take to prevent or delay kidney failure.

Hispanic Outreach: The NKDEP recently launched an effort to raise awareness in Hispanic/Latino audiences about risk factors for chronic kidney disease by developing and disseminating a new Spanish-language brochure, creating new Spanish pages on the NKDEP website, and distributing public service announcements to Spanish radio stations nationwide.

USRDS Data: The NKDEP publicized new data from the NIDDK U.S. Renal Data System (USRDS) that showed that kidney failure rates appear to have stabilized in the past four years. Ongoing promotion of the findings will demonstrate the importance—and benefit—of early detection and proper treatment of chronic kidney disease and the value of programs such as NKDEP.

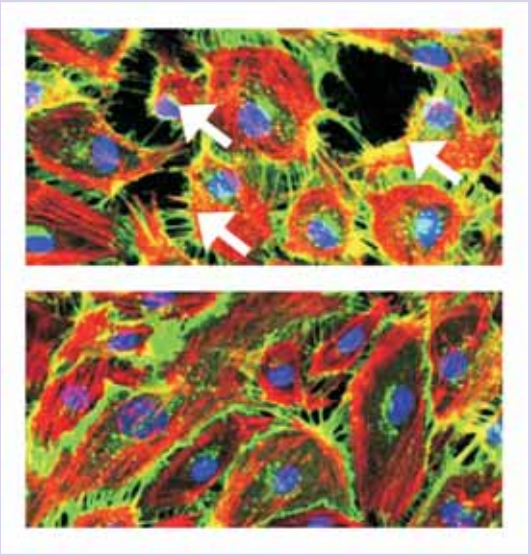
More information about the NKDEP can be found at *http://www.nkdep.nih.gov*

Vignettes in Vascular Biology: Working Across Borders

Dr. Vikas Sukhatme

Dr. Vikas Sukhatme is a leading researcher in the field of vascular biology and kidney disease. His research team focuses on kidney physiology and disease, the mechanisms of cancer growth and spread, the development of preeclampsia and eclampsia, and the regulation of blood vessel growth. Dr. Sukhatme is the Chief of the Renal Division of Beth Israel Deaconess Medical Center and Victor J. Aresty Professor of Medicine at Harvard Medical School and Beth Israel Deaconess Medical Center. The following are scientific highlights based on a scientific presentation Dr. Sukhatme gave to the Institute's National Advisory Council in September 2005.

Dr. Vikas Sukhatme shared several vignettes of "translational vascular biology research in action" to illustrate the insights and advances that can be achieved when scientists work across disciplines when conducting research. Dr. Sukhatme told the Council that previous conceptions of translational research focused primarily on moving research advances from the laboratory into improvements in clinical practice, so-called "bench-to-bedside" translation. In recent years, however, physicians and scientists have come to appreciate that observations and data obtained in a clinical setting have the potential to inform and enrich basic laboratory studies. With this evolution in thinking, translational research can now be envisioned as "bedside-to-bench-and-back" as well. It is thus a bi-directional, dynamic process that is central to the research enterprise and requires close collaboration between basic and clinical researchers. This bi-directional paradigm also highlights the important role that patients play as partners in the biomedical research enterprise.



Sepsis is a bacterial infection that has entered the bloodstream, and can lead to fluid accumulation in the lungs. Normally, the tight association between cells prevents this leakage, but these junctions weaken in patients who have sepsis. Serum from a patient with severe sepsis causes gaps to form between cultured cells (top; black spaces and white arrows). Serum from this patient does not damage the cells if a factor called angiopoietin 2 is first neutralized (bottom).

Image courtesy of S. Parikh and T. Mammoto (Sukhatme Laboratory).

Fundamentals of Vascular Biology

The circulatory system provides nourishment to, and removes wastes from, all the cells of the body. Arteries carry oxygenated blood away from the heart and lungs, while veins return blood laden with carbon dioxide. Arteries and veins are basically long, branching tubes that gradually narrow as distance from the heart

increases. These vessels are connected by a web-like structure of much smaller, narrower vessels called capillaries. It is in the capillaries that oxygen and carbon dioxide are exchanged inside tissues.

In the filtering units of kidneys, capillary beds also unload many other waste products to be excreted in urine. Thus, proper function of the vasculature is critical to obtaining oxygen and removing potentially harmful substances from the body.

Key players in vascular biology are endothelial cells that line the blood vessels, smooth muscle cells that surround the vessels, and circulating cells within the vessels. Dysfunction among any of these cell types can cause disruption of the circulation and its many important functions. Dr. Sukhatme noted that a person who weighs 70 kg—about 150 lbs.—has about 1 kg of endothelial cells in his body. Although this may not sound like a lot, if lined up end-to-end, a person's endothelial cells would stretch 100,000 miles; if laid flat, they would cover over 1000 square meters. Clearly, the vasculature represents a vast system of critical importance in the maintenance of health, and endothelial cells play critical roles in hemostasis, vascular permeability, angiogenesis (sprouting of new vessels from existing ones) and blood pressure control.

The Puzzle of Preeclampsia

During a normal pregnancy, arteries in the mother's uterus undergo changes, or remodeling, in order to ensure an adequate blood supply to the placenta and fetus. In preeclampsia, the pregnant mother's arteries fail to remodel appropriately, resulting in diminished blood supply to the growing fetus. Patients with preeclampsia have high blood pressure and protein in the urine, a sign of kidney damage. However, the underlying causes of preeclampsia have been largely unknown.

Using gene profiling techniques, Dr. S. Ananth Karumanchi, who had previously trained in Dr. Sukhatme's lab and had assumed a faculty appointment in Dr. Sukhatme's division, identified several products that were expressed at high levels in placentas from mothers with the disease. The protein encoded by one of these genes, *sFlt-1*, binds vascular endothelial growth factor (VEGF), a powerful promoter of blood vessel growth. However, sFlt-1 is not capable of transducing the signal carried by VEGF, and thus limits the amount of VEGF available to stimulate cell-bound VEGF receptors. Dr. Karumanchi shared this data with Dr. Sukhatme, who was becoming aware—because of his interest in tumor angiogenesis—that trials of VEGF inhibitors as anti-angiogenic therapy for cancer were producing hypertension and proteinuria as side effects. He suggested to Dr. Karumanchi that sFlt-1 might be involved in preeclampsia, because it would mimic the actions of a VEGF inhibitor. Using a number of animal studies and analyses of patient samples, the researchers showed that diminished VEGF levels, resulting from elevated levels of the sFlt-1 protein, correlated with high blood pressure and kidney damage. Strategies to enhance VEGF signaling, through manipulation of sFlt-1 protein levels, may therefore be a useful avenue to pursue for therapies to prevent or treat preeclampsia. (*For more information about recent advances in preeclampsia research, including Dr. Sukhatme's and Dr. Karumanchi's work, see the Story of Discovery in this chapter.)*

Zebrafish and Endothelial Cell-Target Discovery

The circulatory system appears early in embryonic development. In order to identify potential future targets for therapies of circulatory problems, Dr. Sukhatme and his collaborators looked for genes involved in the development of the vasculature. For these studies, the scientists chose as a model system the zebrafish. This organism is particularly well-suited for several reasons. First, it develops its internal organs over two to five days, allowing rapid screening of a large number of organisms in a relatively short period of time. Second, at early stages of development, it is transparent, allowing developmental progress or problems to be readily observed. To discover genes involved in vasculogenesis, the researchers screened for genes that were expressed

specifically in vessels and then proceeded to use an approach to knock down their expression. Some of animals so treated exhibited defects in blood vessel formation. Several novel genes have been identified by this process and their function and mechanisms of action, as well as their role in human vascular disorders, are under investigation. One gene encoded a protein that seems to be important in embryonic vascular development and, interestingly, this gene is also active at sites of active blood vessel growth in adults. While the size and simplicity of zebrafish impose some limits on these types of studies, this model system represents a useful way to identify potential targets for later, more in-depth investigation.

Vascular Leak—From the Kidney to the Lung

Sepsis is a bacterial infection that has entered the bloodstream, and can lead to excessive fluid in the lungs. If the tiny air sacs within the lungs fill with fluid, the lungs cannot exchange blood-borne carbon dioxide for oxygen. This in turn leads to acute respiratory distress, which can, in extreme cases, result in death.

Normally, the tight association between the cells lining blood vessels prevents leakage of fluid or blood cells from within the vessels into the surrounding tissue. Dr. Sukhatme's team of researchers has identified proteins important to maintaining this leak barrier in the lungs. These proteins are angiopoietin 1 and angiopoietin 2 and their common receptor, TIE-2. Their studies suggest that angiopoietin 1 promotes vessel stability, while angiopoietin 2 promotes vascular permeability in the lung. Vascular leak may therefore be a consequence of an imbalance between angiopoietin 1 and angiopoietin 2.

Support for this hypothesis comes from the observation that acute respiratory distress is often accompanied by a three- to five-fold increase in angiopoietin 2, and that angiopoietin 2 levels return to normal when patients recover. Furthermore, in cultured cells, addition of angiopoietin 2 to the growth medium caused gaps to form in an otherwise tightly associated layer of cells. These findings point to a disturbance in the delicate balance between angiopoietin 1 and 2 as a key factor in the development of vascular leak and respiratory distress. Investigators are examining strategies to block angiopoietin 2 action as potential treatments for sepsis-related vascular leak in humans.

Dialysis Vascular Access: Combating Stenosis

Patients receiving hemodialysis to treat their kidney failure undergo a surgical procedure to create an easily accessible site at which blood will be removed and returned. The creation of this "vascular access graft" facilitates the repeated insertions of relatively wide-diameter needles, because large volumes of blood must be processed to remove toxins no longer filtered out by the patient's kidneys.

Unfortunately, repeated needle punctures often lead to the development of smooth muscle-like lesions in the vascular access grafts. These lesions can cause the graft to narrow, a phenomenon known as "stenosis," and ultimately to fail. They can be treated, but often unsuccessfully.

To try to prevent or treat vascular-access stenosis, Dr. Sukhatme's research team used a pig model to study the molecular factors involved in this process. They found that receptors for platelet-derived growth factor (PDGF) were activated in these lesions, suggesting that agents that block PDGF receptor action might be a valid therapeutic approach. Several such medications are already on the market, though they are not being used currently to treat this condition. One challenge is to devise ways to use such agents locally in relatively high doses, where their activity could be concentrated at the site of the stenosis. Dr. Sukhatme and his colleagues are currently working to address this limitation to current therapy.

Conclusion

Dr. Sukhatme closed by reviewing the long and often arduous path of drug development. This path ranges from the early identification of possible targets, through pre-clinical testing and compound design and development, onward to clinical trials to show effectiveness, and finally, to regulatory approval. He estimated that for every single drug approved for use in patients, 5,000 to 10,000 initial compounds are screened. Studies such as Dr. Sukhatme's vascular development work in zebrafish might help identify potentially beneficial compounds early, and thereby allow researchers to focus their initial studies on the most promising biological targets. Dr. Sukhatme's work on preeclampsia and sepsis illustrates the importance of identifying molecular factors that may play a role in predicting or diagnosing a condition, as they may also be valuable therapeutic targets. Finally, his work on vascular access stenosis illustrates how uncovering a new molecular role for a pathway already targeted in other disease processes may permit the use of existing agents to jump-start research into new therapies. By casting his net widely, Dr. Sukhatme showed how working across scientific borders can strengthen and enlighten all aspects of research.

Helping Women Have a Safer Pregnancy — Advances in Detecting Preeclampsia

A series of research findings may help women avoid a common and sometimes serious complication of pregnancy called toxemia or preeclampsia. This condition usually involves a combination of high blood pressure and persistent swelling, as well as protein in the urine—a sign of impaired kidney function. Preeclampsia can impede blood flow to the baby and result in low birth weight and even graver problems for mother and child. Insights into this condition have been gained by recruiting patients as research partners and combining cutting-edge technology with careful laboratory studies. Moving from the "bench-to-bedside-and-back," investigators used patient samples to design laboratory studies, and then returned to patient data to confirm hypotheses. In doing so, they identified a perturbation in a signaling pathway that may play a central role in preeclampsia. In addition, they developed an assay that may predict preeclampsia with a greater degree of precision than previously. This research has also identified potential targets for new prevention-oriented strategies.

Preeclampsia is a relatively common complication of pregnancy, especially in first pregnancies or in twin pregnancies. The central lesion in preeclampsia is the failure of maternal arteries at the uterus/placenta interface to remodel appropriately. This results in diminished blood supply to the placenta and fetus. Preeclampsia is characterized by high blood pressure and kidney damage resulting in proteinuria, or protein in the urine. It appears in 2.5 to 3.0 percent of pregnancies. Unaddressed, it may progress to eclampsia—violent seizures that can result in the death of the mother and developing child. Children of mothers with preeclampsia may be born prematurely and/or may be small for their age. Treatment for preeclampsia is often not satisfactory. It is managed by close observation of the mother and the administration of anti-hypertensive drugs to lower blood pressure. If the condition progresses, the only effective therapy is urgent delivery of the fetus. Doctors have long believed that placental factors are central to the development of preeclampsia, because the presence of a placenta is an absolute requirement for preeclampsia, and the condition markedly and rapidly improves after delivery.

To investigate possible genetic factors involved in preeclampsia, a research team looked for changes in gene expression in the placentas of women with this condition. They found increased expression of the gene *sFlt-1*, a finding that was interesting for a number of reasons. First, the protein encoded by the *sFlt-1* gene can bind two important growth factors, placental growth factor (PlGF) and vascular endothelial growth factor (VEGF). VEGF and PlGF are powerful promoters of new blood vessel growth, and they play an important role in ensuring the maintenance and survival of the endothelial cells lining blood vessels. Swollen, damaged endothelial cells are one consequence of preeclampsia. Second, the sFlt-1 protein is not anchored in the cell membrane, but circulates in the blood. Although this protein is produced locally in the placenta, it has the potential to act systemically throughout the body. Thus, this protein's ability to bind VEGF and PlGF diminishes the amount of VEGF and PlGF available to endothelial cells. The scientists hypothesized that depletion of VEGF and PlGF due to *sFlt-1* overexpression by the placenta in affected women is a potential explanation for the systemic blood vessel dysfunction that is a hallmark of preeclampsia.

In order to determine whether excessive *sFlt-1* expression might contribute to the vascular derangement seen in preeclampsia, the investigators used laboratory studies to determine the effect of serum from normal and preeclamptic women on the growth of blood vessel cells. Serum from normal women promoted the development of vessel-like tubules in culture, but serum collected from preeclamptic women before delivery inhibited formation of these structures. Intriguingly, when the cells were incubated with serum collected from preeclamptic women 48 hours after delivery, tubules did form. This rapid loss of the unknown factors causing inhibition of tubule formation strongly suggested the involvement of circulating, placenta-derived factors. Furthermore, when researchers induced overexpression of the *sFlt-1* gene in pregnant and non-pregnant rats, both developed hypertension and proteinuria, and their kidneys showed damage remarkably similar to that seen in humans with preeclampsia. Together, these observations pointed strongly toward the involvement of the sFlt-1/VEGF/PlGF signaling pathway in the vascular and kidney complications of preeclampsia.

Going from the "bench-to-the-bedside," the scientists next compared their laboratory results with patient data. They analyzed blood samples from 120 preeclamptic women and 120 normal controls, which had been collected as part of an earlier study. They found that circulating levels of the sFlt-1 protein increased and PlGF levels decreased late in pregnancy in normal women. These changes occurred earlier and were greater in the women in whom preeclampsia developed, and the increase in levels of the sFlt-1 protein preceded the onset of preeclampsia by about five weeks. Building on this finding, researchers turned to urine samples from the same patients. The sFlt-1 protein cannot be measured in urine because the molecule is too large to be excreted intact. Moreover, deducing circulating levels of VEGF in blood using urine samples is problematic, because kidney cells normally secrete VEGF. The researchers therefore measured PlGF protein levels as a surrogate marker of sFlt-1 and VEGF signaling activity because all three share the same pathway. They reported that PlGF protein levels in urine were similar and rising in both groups early in pregnancy. Women who would go on to develop preeclampsia saw this increase slow at around 25 weeks. After the onset of preeclampsia, PlGF protein levels in affected women plummeted to just one-seventh those seen in normal women. Thus, low urinary PlGF protein levels early in pregnancy may be an early warning sign of subsequent preeclampsia.

The ability to measure a factor in urine that may predict preeclampsia represents a significant advance as a diagnostic tool, because no such test previously existed and because urine can be sampled more easily than blood. The VEGF/PlGF signaling pathway also presents multiple potential new targets for developing therapies aimed at preventing or treating preeclampsia. The rapid pace of recent progress in this area gives hope to at-risk women and their children that, through continued research, they may be able to avoid the perils of preeclampsia.

Frankie Cervantes

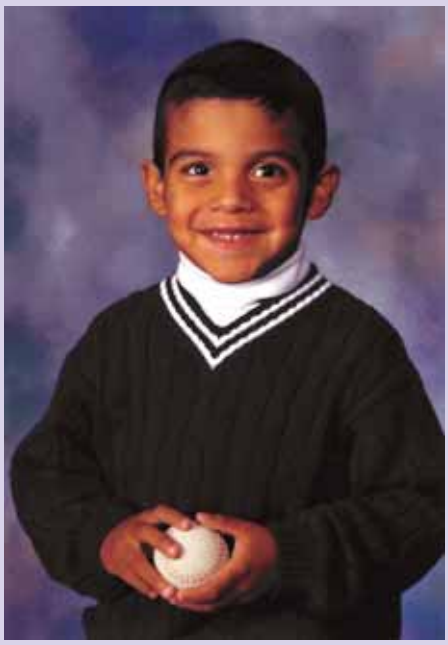
Battling Focal Segmental Glomerulosclerosis: A Little Boy With a Big Wish

Six-year-old Frankie Cervantes awoke extra early the morning he was scheduled for his kidney transplant. He simply could not contain his excitement and joy. "It's kidney day. It's kidney day," Frankie laughingly kept telling his mother and father. "He was so happy," his father says.

And Frankie had every right to be.

At just 18 months of age, Frankie was diagnosed with nephrotic syndrome—symptoms of kidney malfunction—caused by focal segmental glomerulosclerosis (FSGS), a serious kidney disease. By the time he was three years old, the disease had become so severe that Frankie's kidneys failed and needed to be removed to prevent life-threatening complications. Three years later, in August 2005, Frankie was transplanted with a kidney donated by his mother. Despite the successful transplant, the last several years have been extremely hard on the Cervantes family, and Frankie's medical future remains uncertain.

Since his diagnosis, Frankie has experienced several long-term hospitalizations; been through at least two life-threatening experiences; taken numerous medications; and been on extremely restrictive diets. Prior to his transplant, he underwent invasive medical treatment at home every day for more than two and a half years to substitute for lost kidney functions. Then, he experienced a relapse of nephrotic syndrome just days after he received his mother's healthy kidney. Yet Frankie remains a joyful, playful child who loves dinosaurs and toy racing cars—while his parents remain hopeful.



Frankie Cervantes

This is the story of a little boy whose kidneys may have failed him, but not his heart nor spirit, nor the heart, spirit and tenacious love of his mother and father who are counting on medical research to help their son and others like him.

About the Kidneys and Kidney Damage

The kidneys are two bean-shaped organs, located below the ribs toward the middle of the back. The kidneys perform several critical functions in the body. They remove extra water and wastes from the blood, converting them to urine. At the same time, they must ensure that critical blood components (such as blood proteins) do not leak into the urine. The kidneys also keep a stable balance of salts (primarily

sodium and potassium salts) and other substances in the blood, and produce hormones that help build strong bones and help form red blood cells.

If the kidneys become damaged by disease, patients can develop a condition called nephrotic syndrome. Nephrotic syndrome is marked by several symptoms: high levels of protein in the urine (proteinuria); low levels of protein in the blood; swelling (edema), especially around the eyes, feet, ankles, and hands; and high cholesterol. When a patient develops nephrotic syndrome, physicians need to determine what is causing the kidney damage—for example, a chronic disease or an infection—so that they can take appropriate steps in order to halt these potentially life-threatening symptoms.

What is FSGS?

FSGS, or focal segmental glomerulosclerosis, is a medical term that describes scarring in scattered regions of the kidneys. Patients with FSGS experience damage and eventual scarring in the tiny filtering units of the kidneys, called glomeruli. Each kidney has about one million glomeruli, which filter the blood repeatedly—the equivalent of about 200 quarts of blood a day—to remove waste products and extra water to form urine. When the glomeruli are damaged in FSGS, the filters no longer work properly, and blood proteins begin to leak into the urine. If there is heavy loss of protein, a patient develops nephrotic syndrome. The extensive loss of protein in the urine leads to low blood protein levels. This protein loss then, in turn, causes buildup of fluid outside the circulatory system, resulting in excessive swelling in the face, hands, feet or ankles. FSGS also interferes with the kidneys' ability to clear waste products, which begin to build up to toxic levels in the blood.

In some cases, FSGS leads to end-stage renal disease (ESRD), or irreversible kidney failure. ESRD means that in order for a patient to live, he or she needs help to replace kidney functions that are critical for survival. This help comes either in the form of dialysis or a kidney transplant. Dialysis is a medical treatment that mimics the cleansing activities of the kidneys, and their regulation of salt balance. Patients on dialysis are also commonly treated with medications to reduce health problems associated with irreversible kidney failure, such as anemia and bone loss. They also are given guidance on dietary restrictions and meal planning to help reduce the dangerous build-up of wastes in the blood.

First described in 1957, FSGS is an irreversible disease whose cause is often unknown. It appears to be more prevalent and more severe in African American and, perhaps, in Hispanic American children. Frankie's father is Mexican; his mother is Panamanian. Although steroid therapy is commonly used to treat children with FSGS, approximately 75 percent do not respond to therapy, relapse while on therapy, or relapse when therapy is stopped. In short, no current treatment for nephrotic syndrome caused by FSGS is completely satisfactory. As a result, many children and young adults with this condition are at high risk for kidney failure.

Frankie's Story

Aside from being born during his mother's eighth month of pregnancy, Frankie was a perfectly healthy, bouncy, baby boy. Like all young children, he had his bouts with fevers and colds. When he was 18 months old and began running a fever, accompanied by a cough and runny nose, his mother brought him to a pediatrician. The doctor discovered Frankie also had an ear infection, and treated it with penicillin.

That same night, Frankie's cries woke his parents. Their son's eyes, lips and cheeks were extremely swollen. It was obvious Frankie was in great pain, so Mr. and Mrs. Cervantes rushed him to the nearest hospital emergency room. There, they were told that Frankie had likely suffered an allergic reaction to the penicillin. He was given another antibiotic for the infection and an additional medication to reduce the swelling. However, the swelling got worse, so Frankie's parents took him back to the hospital.

PATIENT PROFILE

After numerous tests, including a biopsy of his kidneys, Frankie was diagnosed with FSGS. He was immediately put on the steroid, prednisone, which helped to reduce the swelling and seemed to stabilize his kidney function. Steroids, such as prednisone, as well as other immunosuppressive drugs, appear to help some FSGS patients by decreasing proteinuria and improving kidney function. However, these medications can also produce severe side effects. These side effects include, but are not limited to, increased blood sugar; bone, muscle and eye problems; increased hair growth; and an inability to fight off infection (immunosuppression). Consequently, these drugs can be used for a limited time only. In addition, such treatments are beneficial to only a minority of those in whom they are tried.

Because of the immunosuppressive nature of prednisone, "every time Frankie passed someone on the street with a cough or a cold, he'd get it," says his father. Frankie was on prednisone for a year. However, as physicians began to reduce his dosage, Frankie's swelling increased again, as did his pain.

The Cervantes family struggled to find treatments that could help alleviate Frankie's symptoms, including alternatives to standard medical therapies, such as acupuncture. Frankie's condition only worsened. Mr. and Mrs. Cervantes relate how, one morning, Frankie woke up crying in such pain that he began pulling at his mother's hair and pleaded to be brought back to the hospital. He was immediately treated again with prednisone and remained in the hospital for three months. This time, however, he did not respond to the prednisone treatment.

Blood tests and a kidney biopsy showed that Frankie was suffering from a severe case of FSGS, and his kidneys were shutting down. He was dialyzed to clear waste products. Because his kidney disease was so severe, then-three-year-old Frankie had his kidneys removed to prevent catastrophic loss of blood proteins and save his life. To stay alive until a kidney donor could be identified and Frankie's body was mature enough to accept a transplant, his mother was trained to perform one type of dialysis procedure, called peritoneal dialysis, at home. The procedure required her to administer this 11-hour blood-cleansing procedure seven days a week to her 3-year-old, hard-to-sit-still son, mostly at nighttime, when Frankie was asleep. Moreover, Frankie had to take medications that could help substitute for some of the other kidney functions he had lost. For example, with his family's approval, Frankie was given a medication to try to control hyperparathyroidism (high parathyroid hormone). This condition develops as a result of kidney failure and causes bone loss. Frankie's father understates it when he says, "It's been hard."

The painstaking task of almost daily dialysis went on for two years and seven months. In that time, Frankie contracted an infection that landed him in the hospital yet again, this time for a month, two weeks of which were spent in the intensive care unit. "We almost lost him," says an emotional Mr. Cervantes.

The good news was that also during this time it was discovered that Mrs. Cervantes was eligible to donate one of her kidneys to her son. "I could not have been happier to learn that we matched," she says with a big smile, as she holds her son on her lap.

The Transplant

The family needed to wait nearly a year before Frankie's body matured enough to accept his mother's kidney. During that time, Frankie remained on a strict diet in order to control his blood pressure and cholesterol levels. "No milk, no fruit, no salt, no potassium, no nothing," says Mr. Cervantes. "Frankie would see kids eating ice cream and chips. It was very hard for us to always tell him 'no'."

When the day finally came for Frankie to receive his mother's kidney, Frankie, who had been through so much so young, was joyful. When he walked by the hospital security station he cheerily said to the guard on duty, "I'm getting my new kidney today."

The new kidney worked perfectly for three days. Then, tests began to show the return of excessive amounts of protein in Frankie's urine, a sign that his FSGS was recurring and inducing nephrotic syndrome. He was plasmapheresed, a medical treatment whereby the blood is treated outside the body to remove harmful factors, and then returned to the patient. Fortunately, Frankie responded well to the treatment. But after all they've been through, Mr. and Mrs. Cervantes feel they are living with a "time bomb." "We never know when this disease will explode again," says Mr. Cervantes.

Hope Through Research

The NIDDK is seeking to defuse the time bomb of disease for Frankie and other children with FSGS. Working with the American Society of Pediatric Nephrology, the NIDDK has formed a collaborative network of research centers to conduct a clinical trial for treatment of FSGS in children and young adults. The goal of the trial is to compare the effectiveness of two treatment regimens in reducing proteinuria in patients who, like Frankie, have steroid-resistant FSGS of unknown cause, but who have not yet had a kidney transplant. Moreover, NIDDK scientists are committed to studying possible causes of various forms of FSGS. These researchers are conducting clinical studies of therapeutic approaches that may prevent recurrence in transplant patients, as well as additional studies of treatments for FSGS in patients.

Because of the immunosuppressant medications Frankie is currently taking to help prevent his body's rejection of his new kidney, he must limit his exposure to germs. For now, Frankie—who is a first-grader—is being taught by a teacher who comes to his home. Like all little boys his age, Frankie loves to play video games, ride his bike and watch family movies. When asked what his one big wish is, Frankie says "to swim in the ocean"—a dream that has been difficult to attain for the past few years because of his peritoneal dialysis treatment. Now, with his new kidney, Frankie may one day be granted his wish.

For more information on the FSGS Clinical Trial, see *http://www.clinicaltrials.gov/ct/show/NCT00135811?order=8*

Frankie Cervantes

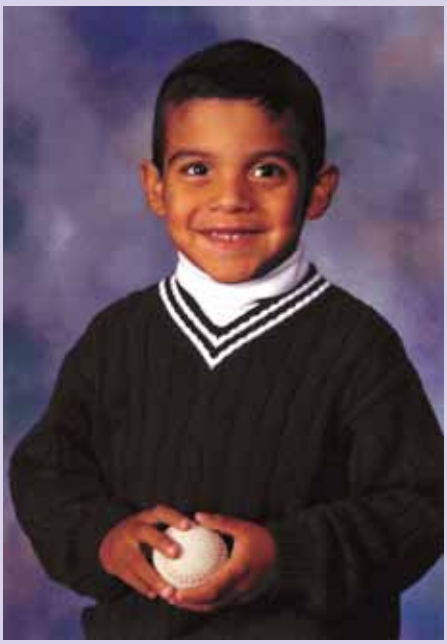
Una Lucha contra la Glomeruloesclerosis Focal y Segmentaria: Un Chiquitín con un Gran Deseo

Frankie Cervantes, un niño de seis años, se despertó mucho más temprano de lo habitual la mañana en que su trasplante de riñón estaba programado. Simplemente no podía contener su entusiasmo y alegría. "Es el día del riñón. Es el día del riñón," Frankie reía al decírselo una y otra vez a su madre y a su padre. "Estaba tan contento," comenta su padre.

Realmente, Frankie tenía todo el derecho a estar feliz.

A tan sólo 18 meses de edad, a Frankie se le diagnosticó síndrome nefrótico, un cuadro de insuficiencia renal, causado por la glomeruloesclerosis focal y segmentaria, una grave enfermedad del riñón. Cuando Frankie cumplió tres años, la enfermedad había empeorado tanto que los riñones de Frankie dejaron de funcionar y fue necesario extirparlos para prevenir complicaciones potencialmente mortales. Frankie sobrevivió durante este periodo gracias al uso de diálisis renal. Varios años más tarde, en agosto de 2005, a Frankie se le trasplantó un riñón donado por su madre. A pesar del éxito del trasplante, los últimos años han sido extremadamente difíciles para la familia Cervantes, y el futuro médico de Frankie es todavía incierto.

Desde su diagnóstico, Frankie ha sido hospitalizado muchas veces por largos períodos; ha tenido por lo menos dos experiencias que amenazaron su vida; ha tomado un sin fin de medicamentos y ha participado en regímenes alimenticios extremadamente complejos. Antes de su trasplante, se le administró diariamente en su casa, por más de dos años y medio, un tratamiento médico agresivo para sustituir las funciones renales perdidas. Luego, él experimentó una



Frankie Cervantes

recaída cuando el síndrome nefrótico se presentó tan sólo unos cuantos días después de haber recibido el riñón sano de su madre. Aún así, Frankie continúa siendo un niño juguetón y feliz, que ama a los dinosaurios y a los cochecitos de carreras, mientras que sus padres no pierden la esperanza que su condición médica mejore.

Esta es la historia de un chiquitín cuyos riñones fallaron, pero no su corazón ni su espíritu, ni el corazón ni el espíritu y el firme amor de su madre y su padre, quienes tienen fe en que las investigaciones médicas podrán ayudar a su hijo y a otros que padecen esta enfermedad.

Información sobre los Riñones y el Daño Renal

Los riñones son dos órganos en forma de frijol (habichuela), ubicados en el abdomen debajo de las costillas hacia la parte media de la espalda. Los riñones desempeñan varias funciones vitales en el cuerpo. Extraen el exceso de agua y los desechos de la sangre para convertirlos en orina. Al mismo tiempo, deben asegurar que los componentes vitales de la sangre (tales como las proteínas que residen en ella) no pasen a la orina. Los riñones también mantienen un equilibrio estable de sales (principalmente las sales de sodio y potasio) y de otras sustancias en la sangre, y producen hormonas que ayudan a formar huesos fuertes y también glóbulos rojos (eritrocitos).

Si alguna enfermedad daña a los riñones, los pacientes pueden contraer una enfermedad denominada síndrome nefrótico. El síndrome nefrótico se caracteriza por varios síntomas: altas concentraciones de proteína en la orina (proteinuria); bajas concentraciones de proteína en la sangre; hinchazón (edema), especialmente alrededor de los ojos, los pies, tobillos y manos; y alto colesterol. Cuando un paciente padece un síndrome nefrótico, los médicos necesitan determinar qué es lo que está causando el daño a los riñones, por ejemplo, una enfermedad crónica o una infección, de manera que puedan tomar los pasos adecuados para detener estos síntomas que son potencialmente mortales.

¿Qué es la Glomeruloesclerosis Focal y Segmentaria?

La glomeruloesclerosis focal y segmentaria (FSGS, siglas en inglés) es un término médico que describe la cicatrización que ocurre en distintas regiones de los riñones. Los pacientes con FSGS experimentan daños y posteriormente cicatrización en las minúsculas unidades de filtración de los riñones, denominadas glomérulos. Cada riñón tiene aproximadamente un millón de glomérulos, los cuales filtran la sangre repetidamente (el equivalente a 180 litros o 50 galones de sangre cada día) para extraer los desechos y el exceso de agua para formar la orina.

Cuando los glomérulos se dañan en la FSGS, los filtros dejan de funcionar correctamente, y las proteínas de la sangre empiezan a pasar a la orina. Si ocurre una gran pérdida de proteínas, el paciente contrae el síndrome nefrótico. La abundante pérdida de proteínas en la orina resulta en bajas concentraciones de proteínas en la sangre. Esta pérdida de proteínas, a su vez, causa una acumulación de líquido fuera del sistema circulatorio, lo que resulta en una hinchazón excesiva de la cara, manos, pies o tobillos. La FSGS también interfiere con la capacidad de los riñones de retirar los desechos, los cuales empiezan a acumularse en cantidades tóxicas en la sangre.

En algunos casos, la glomeruloesclerosis focal y segmentaria es causa de la nefropatía terminal (ESRD, siglas en inglés) o insuficiencia renal irreversible. La nefropatía terminal significa que, para que un paciente pueda vivir, él o ella necesita ayuda para sustituir las funciones renales que son fundamentales para la supervivencia. Esta ayuda se proporciona en forma de diálisis o de un trasplante de riñón. La diálisis es un tratamiento médico que imita las actividades de limpieza de los riñones, así como también su regulación del equilibrio de las sales. Los pacientes sometidos a diálisis también son tratados comúnmente con medicamentos para disminuir los problemas de salud relacionados con la insuficiencia renal irreversible, tales como la anemia y la osteopenia (debilitamiento de los huesos) y la hipertensión (alta presión sanguínea). Además, se les brinda orientación respecto a restricciones alimenticias y planificación de comidas para ayudarlos a reducir la peligrosa acumulación de desechos en la sangre y limitar la ingesta de sal.

La glomeruloesclerosis focal y segmentaria, que fuera descrita por primera vez en 1957, es una enfermedad irreversible cuya causa es con frecuencia desconocida. Parece ser más común y más grave en niños afroamericanos y, quizás también, en niños hispanos. El padre de Frankie es de México, y su madre es de Panamá. Aunque el tratamiento con medicinas llamadas esteroides se utiliza comúnmente para tratar a

niños con FSGS, aproximadamente el 75 por ciento de ellos no reacciona favorablemente al tratamiento, recae durante el tratamiento o recae cuando se detiene el tratamiento. En pocas palabras, no se cuenta actualmente con un tratamiento para el síndrome nefrótico causado por la FSGS que sea completamente satisfactorio. Como resultado, muchos niños y adultos jóvenes que padecen esta enfermedad corren un gran riesgo de desarrollar insuficiencia renal.

La Historia de Frankie

A pesar de haber nacido durante el octavo mes de embarazo de su madre, Frankie era un bebé lleno de vida y perfectamente sano. Como todos los niños pequeños, tuvo sus episodios de fiebres y resfriados. Cuando a los 18 meses de edad se le presentó una fiebre acompañada con tos y goteo nasal, su madre lo llevó a un pediatra. El médico determinó que Frankie también tenía una infección del oído y le dió tratamiento con penicilina.

Esa misma noche, los llantos de Frankie despertaron a sus padres. Los ojos, labios y mejillas de su hijo estaban extremadamente hinchados. Era obvio que Frankie tenía mucho dolor, así que los Cervantes lo llevaron rápidamente a la sala de emergencias del hospital más cercano. Ahí, les informaron que Frankie muy probablemente había tenido una reacción alérgica a la penicilina. Le administraron otro antibiótico para la infección y un medicamento más para disminuir la hinchazón. Sin embargo, la hinchazón empeoró, así que los padres de Frankie lo llevaron de regreso al hospital.

Tras numerosas pruebas, entre ellas una biopsia de los riñones, a Frankie se le diagnosticó glomeruloesclerosis focal y segmentaria. Inmediatamente se le administró prednisona, un esteroide, que lo ayudó a disminuir la hinchazón y que aparentemente estabilizó la función de sus riñones. Como mencionáramos previamente, los esteroides, tales como la prednisona, así como otros medicamentos inmunodepresores, parecen ayudar a algunos pacientes que padecen FSGS al disminuir la proteinuria y mejorar el funcionamiento de los riñones. No obstante, estos medicamentos pueden también producir efectos secundarios graves. Entre estos efectos secundarios se incluyen, por ejemplo, aumento de azúcar en la sangre (hiperglucemia); problemas de los huesos, músculos y ojos; aumento en el crecimiento de vello corporal; e incapacidad para luchar contra las infecciones (inmunodepresión). Por consiguiente, estos medicamentos sólo pueden utilizarse por un tiempo limitado. Además, dichos tratamientos son beneficiosos solamente para un pequeño porcentaje de aquellos que los reciben.

Debido a la naturaleza inmunodepresora de la prednisona, “cada vez que Frankie pasaba junto a alguien en la calle que tuviera tos o un resfriado, él se contagiaba,” relata su padre. Frankie fue tratado con prednisona por un año. Sin embargo, a medida que los médicos empezaron a reducir su dosis, la hinchazón de Frankie y su dolor aumentaron nuevamente.

La familia Cervantes luchó para encontrar tratamientos que pudieran ayudar a aliviar los síntomas de Frankie, entre ellos, tratamientos distintos a los tratamientos médicos normales, como por ejemplo la acupuntura. La enfermedad de Frankie sólo empeoró. El Sr. y la Sra. Cervantes cuentan como una mañana, Frankie se despertó llorando con tanto dolor que empezó a jalarle el cabello a su madre y le rogó que lo llevara de regreso al hospital. Fue tratado nuevamente con prednisona, y permaneció hospitalizado durante tres meses. En esta ocasión, sin embargo, no reaccionó favorablemente al tratamiento con la prednisona.

Los análisis de sangre y una biopsia renal mostraron que Frankie padecía un grave caso de glomeruloesclerosis focal y segmentaria, y que sus riñones estaban dejando de funcionar. Fue sometido a diálisis para eliminar los desechos. Debido a que su caso de nefropatía era tan grave, a Frankie, quien apenas tenía tres años de edad, se le extirparon sus riñones para prevenir una pérdida catastrófica de proteínas de la sangre y salvar su vida. Para mantener vivo a Frankie

hasta que se pudiera identificar un donante de riñón y que su cuerpo estuviera lo suficientemente maduro para aceptar un trasplante, se capacitó a su madre para realizar en casa un tipo de procedimiento de diálisis, llamado diálisis peritoneal. Se requería que siete días a la semana ella administrara a su hijo inquieto, de tres años de edad, este procedimiento de limpieza de sangre de 11 horas, casi siempre en la noche, mientras Frankie estaba dormido. Además, Frankie tuvo que tomar numerosos medicamentos para ayudarlo a sustituir algunas de las otras funciones renales perdidas. Por ejemplo, con la aprobación de su familia, a Frankie se le administró un medicamento para intentar controlar el hiperparatiroidismo (producción excesiva de la hormona paratiroidea). Esta enfermedad se presenta como resultado de la insuficiencia renal y es causa de fragilidad en los huesos (osteopenia). Las palabras del padre de Frankie no reflejan plenamente la realidad cuando él dice, "ha sido una experiencia difícil."

La tarea meticulosa de la diálisis casi diaria fue realizada por casi tres años. En ese entonces, Frankie contrajo una infección que lo forzó a ser hospitalizado una vez más, en esta ocasión por un mes, de la cual dos semanas las pasó en una unidad de cuidados intensivos. "Casi lo perdimos," cuenta sentimentalmente el Sr. Cervantes.

La buena noticia es que también durante ese tiempo se determinó que la Sra. Cervantes era una persona apta para donar uno de sus riñones a su hijo. "No pude haber estado más contenta cuando me enteré de que era un donante adecuado para mi hijo," cuenta con una gran sonrisa, con su hijo sentado en sus piernas.

El Trasplante

La familia necesitaba esperar casi un año antes de que el cuerpo de Frankie estuviera lo suficientemente maduro para aceptar el riñón de su madre. Durante ese tiempo, Frankie permaneció en un régimen alimenticio estricto a fin de controlar su presión arterial y las concentraciones de colesterol. "Nada de leche, fruta, sal, ni potasio; nada de nada," dice el Sr. Cervantes. "Frankie veía a otros niños comer helado y papitas fritas. Era muy difícil para nosotros tener que decirle siempre que 'no'."

Cuando finalmente llegó el día en el que Frankie recibiera el riñón de su madre, Frankie, quien había padecido tanto siendo tan pequeño, estaba feliz. Al pasar por la estación de seguridad del hospital le dijo entusiasmado al guardia de turno, "hoy me van a dar mi riñón nuevo."

El riñón nuevo funcionó perfectamente durante tres días. Luego, las pruebas empezaron a mostrar la recurrencia de cantidades excesivas de proteína en la orina de Frankie, una señal de que la glomeruloesclerosis focal y segmentaria estaba presentándose nuevamente y causando síndrome nefrótico. Se le administró un procedimiento de plasmaféresis, es decir, un tratamiento médico mediante el cual se trata la sangre fuera del cuerpo para retirar de ella factores perjudiciales para luego regresarla al paciente. Afortunadamente, Frankie reaccionó bien al tratamiento. Pero después de todo lo que han sufrido, los Cervantes sienten que están viviendo con una "bomba de tiempo." "Nunca sabemos cuándo va a explotar nuevamente esta enfermedad," dice el Sr. Cervantes.

Esperanza que Nace de la Investigación

El Instituto Nacional de la Diabetes y Enfermedades Digestivas y del Riñón (National Institute of Diabetes and Digestive and Kidney Diseases, NIDDK) está interesado en desactivar la bomba de tiempo de enfermedad de Frankie y de otros niños que padecen la glomeruloesclerosis focal y segmentaria. Con ayuda de la Sociedad Estadounidense de Nefrología Pediátrica (American Society of Pediatric Nephrology), el NIDDK ha formado una red de colaboración de centros de investigación para realizar un estudio clínico sobre el tratamiento de FSGS en niños y adultos jóvenes. El objetivo del estudio clínico es comparar la eficacia de dos tratamientos para disminuir la proteinuria en pacientes que, como Frankie, padecen una forma de FSGS de causa desconocida que es

RESEÑA DE UN PACIENTE

resistente a los esteroides, pero quienes todavía no han tenido un trasplante de riñón. Asimismo, los científicos del NIDDK están comprometidos a estudiar las posibles causas de otras formas de la FSGS. Estos investigadores están realizando estudios clínicos de enfoques terapéuticos que pudieran prevenir la recurrencia en pacientes que han recibido trasplantes, así como también otros estudios de tratamientos para la FSGS en estos pacientes.

Debido a los medicamentos inmunodepresores que Frankie está tomando actualmente para ayudar a prevenir que su cuerpo rechace su nuevo riñón, él debe limitar su exposición a microbios. Por ahora Frankie, que es un estudiante de primer grado, recibe instrucción de un maestro que lo visita en casa. Como todos los niños de su edad, a Frankie le encanta jugar videojuegos, andar en bicicleta y ver películas. Cuando le preguntan cuál es su mayor deseo, Frankie contesta “nadar en el océano,” un sueño que ha sido difícil de lograr durante los últimos años debido a su tratamiento de diálisis peritoneal. Ahora, con su nuevo riñón, es posible que algún día se cumpla el deseo de Frankie.

Para obtener mayor información sobre el estudio clínico de la glomeruloesclerosis focal y segmentaria (FSGS), visite la página *http://www.clinicaltrials.gov/ct/show/NCT00135811?order=8*

ACKNOWLEDGEMENTS

Printed February 2006
For Administrative Use

Research

The NIDDK gratefully acknowledges the contributions of the researchers whose studies are described in this document, and of the patients who participate in clinical research studies.

Writing and Production

Overall Production
David Miller, Ph.D., Office of Scientific Program and Policy Analysis
B. Tibor Roberts, Ph.D., Office of Scientific Program and Policy Analysis

Highlights of Research Advances and Opportunities
Staff of the NIDDK Office of Scientific Program and Policy Analysis
Patrick Donohue, Ph.D.
Richard Farishian, Ph.D.
Carol Feld, M.A.
Lisa Gansheroff, Ph.D.
Shefa Gordon, Ph.D.
Mary Hanlon, Ph.D.
Eleanor Hoff, Ph.D.
David Miller, Ph.D.
Megan Miller, Ph.D.
Anne Montgomery
Sharon Pope, M.S.
B. Tibor Roberts, Ph.D.
Christopher Von Seggern, Ph.D., M.P.H.

Contributions from the NIDDK Office of Communications and Public Liaison
Joan Chamberlain, B.A.
Leslie Curtis, M.A.
Joanne Gallivan, M.S., R.D.
Elisa Gladstone, M.P.H.
Mary Harris
Melissa McGowan, M.H.S.
Marcia Vital, M.S.

Patient Profiles
Larry Checco, Checco Communications
Spanish translation of the Frankie Cervantes patient profile (pp. 72-76) provided by the NIH Library Translation Unit, and reviewed by Joan Chamberlain, B.A. and Saul Malozowski, M.D., Ph.D.

Design
Gregg Deal, the Hill Group

Publication Coordination
the Hill Group

For more information on the NIDDK and its mission, and links to other sites, visit: *http://www.niddk.nih.gov.*


NIDDK