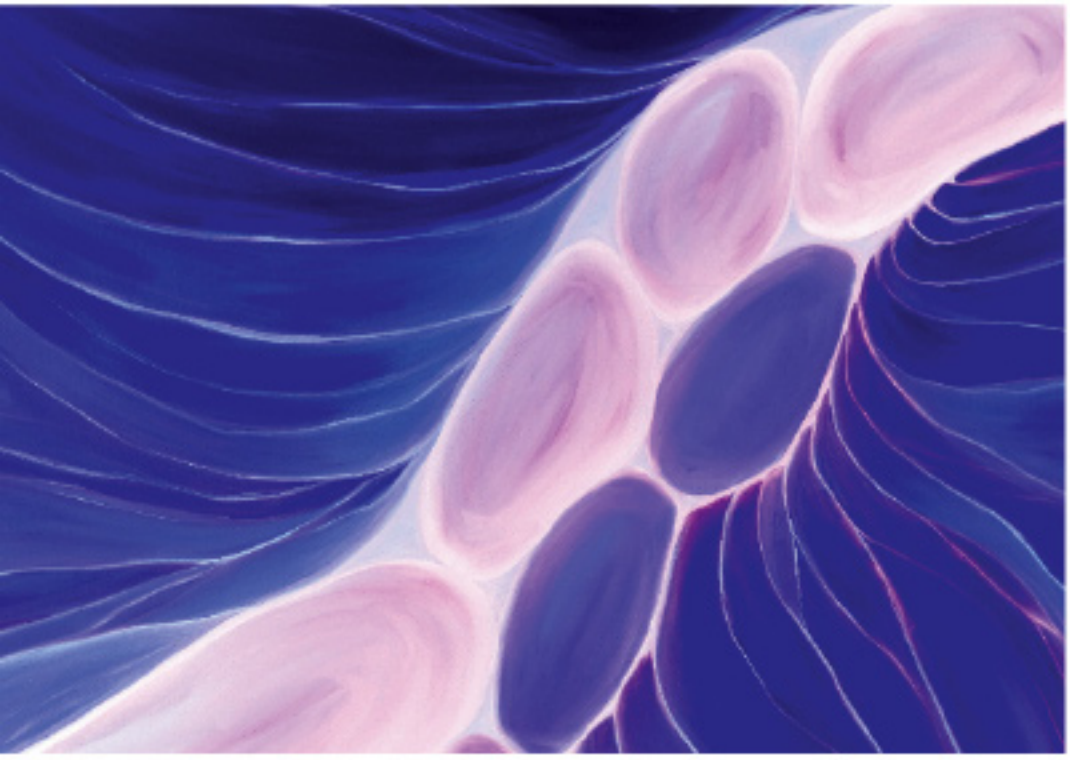
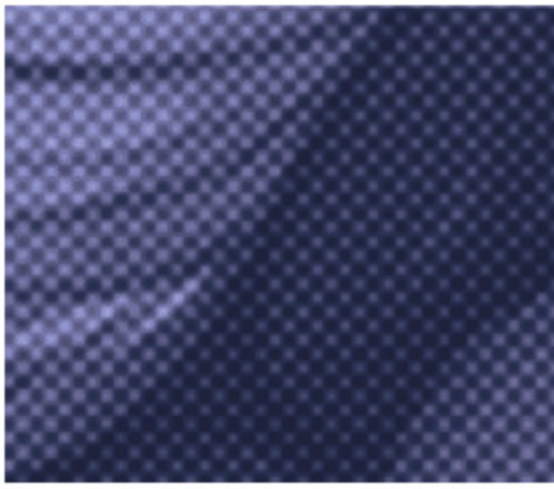
NIDDK

Recent Advances & Emerging Opportunities

February 2007





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

Insights into obesity, diabetes, cancer and other health problems can emerge from investigations into rare diseases. The artwork on the cover represents benign growths—known as hamartomas—that sometimes develop within tissues of people with Tuberous Sclerosis, Peutz-Jeghers Syndrome, and Cowden's Syndrome. The image was adapted from an illustration commissioned for a recent meeting on "Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes," which was cosponsored by NIDDK, NCI and the NIH Office of Rare Diseases. Data presented at the meeting show that the mutations causing these diseases all impinge on a pathway affecting a key molecular regulator that ties protein synthesis, cell growth, and cell division to energy availability. This key regulator is known as mTOR (mammalian Target of Rapamycin), and its signaling pathway is a target for development of therapeutics for many disorders including diabetes, obesity, and cancer. Important new findings that link mTOR to regulation of appetite are discussed in an advance on "New Insights into Molecular Signals that Influence Hunger" in the section of this compendium that addresses obesity.

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MESSAGE FROM THE DIRECTOR

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) supports research on some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases, liver and other digestive diseases, nutritional disorders, obesity, kidney and urologic diseases, and hematologic diseases. Each year, we take time to reflect over the past year's basic and clinical research achievements supported by the NIDDK and to highlight them in this annual compendium, now in its seventh year.

The research advances and programs described herein represent a substantial return on investments made in previous years. Research resources were made possible during the doubling of the NIH budget, including repositories of samples collected for large clinical trials and consortia, such as the IBD Genetics Consortium. Such resources are now enabling pursuit of additional studies, including those aimed at fundamental, cross-disciplinary research questions relating to conditions within the NIDDK mission. We are also strengthening our collaborations with other NIH Institutes and Centers through studies on gene-environment interactions, biomarkers, and nuclear receptor signaling pathways.

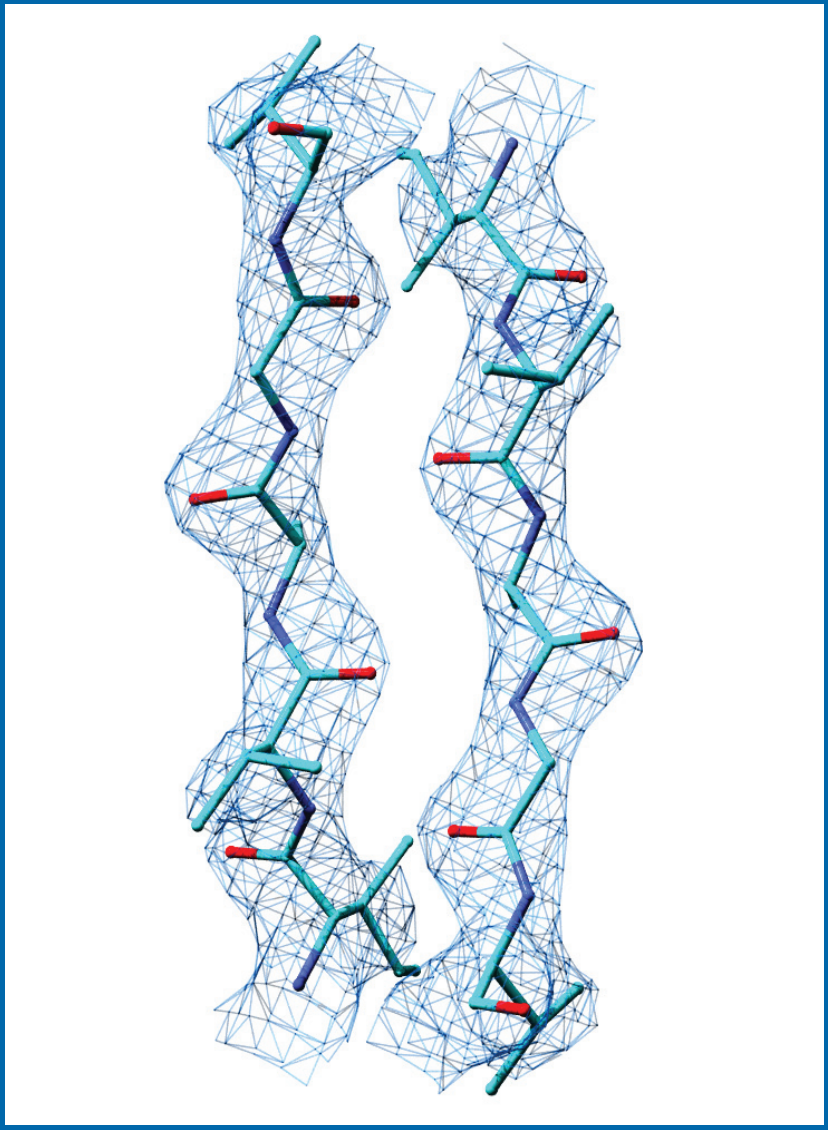
The solid research foundation built through past NIDDK support is bearing fruit in terms of research toward translating basic and clinical research findings to successful interventions. For example, this document presents results of pilot studies for the recently launched HEALTHY trial designed to target type 2 diabetes risk factors in children, as well as continuing benefits from translating findings of the Diabetes Prevention Program to promote healthier lifestyle choices for adults at risk of type 2 diabetes. Furthermore, translational successes are now visible in the development of new clinical tools to improve diagnosis and monitoring of disease. Examples include new imaging techniques for polycystic kidney disease and continuous glucose monitors to assist patients in managing type 1 diabetes.

The NIDDK continues to devote efforts to ensuring that knowledge gained from these major NIDDK-sponsored research advances reaches health care providers and patients. Such efforts include ongoing educational programs, such as the National Kidney Disease Education Program and the National Diabetes Education Program, as well as a new program to promote celiac disease awareness and a women's urologic health outreach program currently under development. This annual publication also aims to further the goal of disseminating to stakeholders information on NIDDK-supported research advances.

By engaging in highly collaborative strategic planning, the Institute endeavors to maximize use of its resources to best support future research advances. Recent efforts reflect collaboration with external experts from the scientific, health care, and patient advocacy communities, as well as research partners from across the NIH and other Federal agencies. For example, the Institute led the development, with broad stakeholder input, of a *Type 1 Diabetes Research Strategic Plan* and a *Strategic Plan for Pediatric Urology.* The Institute is currently providing leadership to the development of a long-range research plan by the National Commission on Digestive Diseases.

This compendium reflects only a fraction of the immense body of work performed by basic scientists, clinical researchers, and patient volunteers. We hope that this booklet conveys a sense of the NIDDK's important contributions to the national biomedical research enterprise.

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



3-D model of a portion of human cystatin C—a serum protein being studied as a potential "biomarker" of impaired renal function. NIDDK-supported efforts to identify and qualify biomarkers for specific diseases within its mission are described in this chapter.

Image courtesy of Dr. Mariusz Jaskolski. Adapted by permission from Macmillan Publishers Ltd: Nature Structural Biology 8: 316-320, copyright (2001).

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, RNA, 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. Translational research also includes efforts to identify and use molecules found in the body, such as gene variants and proteins, as biological markers ("biomarkers") of specific disease states.

ACCELERATING BIOMARKER RESEARCH

A biomarker can be defined as a physical, functional, or biochemical indicator of a physiological or disease process that has diagnostic or prognostic utility. 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. Some biomarkers are extremely valuable surrogate endpoints for diseases, and can be used as outcome measures in clinical trials. The capacity to study and treat disease is hampered when unique, reliable, quantifiable, easily measured, and verified biomarkers that correlate well with disease progression are lacking. Therefore, the NIDDK Translational Research Working Group, charged with identifying opportunities for accelerating the transition of basic biomedical research advances from the laboratory to the common clinical application, has identified biomarker research as a particularly critical area for study.

The NIDDK has a long track record of successfully promoting the development of biomarkers for a number of diseases within its research mission that have transformed patient care. For example, the hemoglobin A1c (HbA1c) blood test has been shown to be a good surrogate measure of long-term blood glucose control in diabetes. HbA1c has been validated in a large NIDDK-funded clinical trial, and subsequently has served as the basis for the Food and Drug Administration's approval of multiple drugs for therapy of diabetes. Similarly, methods for assessing kidney function by estimating glomerular filtration rate (GFR) using circulating levels of the metabolite protein creatinine and earlier ascertainment of kidney disease by measuring the level of the protein albumin in the urine, have become important biomarkers for kidney function and disease. However, additional biomarkers are urgently needed to speed development of potential new treatments. For example, cystatin C is a different serum protein now being investigated as a possible biomarker for estimating GFR.

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.

This year, the Foundation for the National Institutes of Health (FNIH), the National Institutes of Health (NIH), the Food and Drug Administration (FDA), and the Pharmaceutical Research and Manufacturers of America (PhRMA) announced a major public-private biomedical research partnership, *The Biomarkers Consortium*, to search for and validate new biomarkers to accelerate dramatically biomarker usage for early detection, diagnosis, and treatment of disease. Among the first projects to be undertaken by the *Consortium* will be one on diabetes and pre-diabetes biomarkers. This project would build upon an existing NIDDK pilot study, and would seek to discover new biomarkers related to type 2 diabetes and pre-diabetes that could be used in developing a new assay and speed up and improve the translation of this assay from the bench to the bedside. The hope is that this project will lead to a more reliable, economical, and faster diagnostic test for diabetes by identifying biomarkers from before the disease really begins to the time of full-blown diabetes. This would allow earlier treatment, better monitoring, and ultimately reduce morbidity and mortality from diabetes and provide the potential for dramatic savings in health care costs.

Additional new NIDDK-sponsored initiatives to pursue potential biomarkers include:

- *Development of Disease Biomarkers:* Previous studies have identified candidate biomarkers. Other studies test biomarkers in clinical trials. This initiative will fill the gap between these steps by providing resources to demonstrate that candidate biomarkers meaningfully reflect actual disease processes. The initiative's scope includes well-defined human diseases of liver, kidney, genitourinary tract, digestive and hematologic systems, and endocrine and metabolic disorders, diabetes and its complications, and obesity, for which there are no or very few biomarkers, or for which standard biomarkers are currently prohibitively invasive or expensive.

- *Noninvasive Methods for Diagnosis and Progression of Diabetes, Kidney, Urological, Hematological, and Digestive Diseases:* This initiative is similar in scope to the one described previously, but rather than focusing on validation, it emphasizes development of new technologies or new applications of existing technologies, including molecular imaging and functional imaging approaches; imaging methods with high spatial, chemical, or time resolution; and new spectroscopic or sensor array technologies for monitoring metabolic or physiological events.

- *Biomarker Development for Diabetic Complications:* Clinical studies, such as the Diabetes Control and Complications Trial, have demonstrated that tight glycemic control can dramatically reduce the micro- and macrovascular complications of diabetes. However, intensive glycemic control is difficult to obtain in all patients and is associated with an increased risk of hypoglycemia. This initiative seeks biomarkers that can help identify

patients with diabetes who are at particular risk for various comorbidities. Better assessment of the progression of complications may also help to facilitate the development of therapeutics for these devastating conditions.

- *Toward Imaging the Pancreatic Beta Cell in People:* Pancreatic beta cells secrete insulin, which signals other cells in the body to take up glucose from the blood. It is the beta cells that are destroyed during type 1 diabetes. Imaging of those beta cells could allow physicians to follow disease progression, and assess treatments, as well as keep track of new beta cells introduced via transplant. This initiative solicits research applications focused on detection (within the body) of beta cell mass, function, inflammation, or transplanted islet engraftment, especially using imaging technologies. It is intended to support the development of novel imaging technologies that will provide new opportunities for evaluating and quantifying beta cell mass and function with potential to lead to the development of a clinically useful exam.

In addition, the NIDDK is the lead Institute for the *Using Metabolomics to Investigate Biological Pathways and Networks* Request for Applications under the NIH Roadmap for Medical Research. Metabolomics—the study of all metabolites (e.g., salts, sugars, and fats) in order to understand physiological and disease processes—is a relatively new and fertile area of research. This initiative seeks to encourage the use of innovative metabolomics technologies to establish methods and model systems for advancing the understanding of biological pathways and networks, their temporal and spatial resolution, and their regulation in health and disease states. Many of the applications are expected to be relevant to NIDDK mission-specific diseases.

Kidney Disease of Diabetes Project Selected for Whole Genome Association Study

The Genetic Association Information Network (GAIN) is a new public-private partnership of the Foundation for the National Institutes of Health (FNIH) and includes partnerships with the NIH and the private sector to encourage whole genome association studies of common diseases. Using biological samples already collected in clinical studies, GAIN will evaluate the subtle differences between the genomes of people with and without six common diseases that affect the public health. After vigorous peer, technical, and ethical reviews, the Genetics of Kidneys in Diabetes (GoKinD) study was selected as one of the six studies for analysis by GAIN. The NIDDK participates in GoKinD, which is led by the Centers for Disease Control and Prevention and the Juvenile Diabetes Research Foundation International. GoKinD has the largest single collection of biosamples and data for research on the genetic causes of kidney disease in type 1 diabetes. The collection consists of samples from people with both type 1 diabetes and kidney disease, people with type 1 diabetes but no kidney disease, and samples from some of the parents of affected patients. Using the GoKinD collection, investigators will search for genes that predispose patients to, or protect them from, developing this devastating complication of diabetes. This knowledge could accelerate the development of new methods for prevention, diagnosis, and treatment. Data from this effort will be deposited into a central database and made available for broad research use. The GAIN study and the public release of data are helping to ensure the broadest possible access to genetic data from the valuable GoKinD collection.

NUCLEAR RECEPTOR SIGNALING ATLAS (NURSA)

The Nuclear Receptor Signaling Atlas (NURSA) is a trans-NIH initiative, led by the NIDDK, designed to develop a comprehensive understanding of the structure, function, and role in disease of nuclear hormone receptors. Nuclear receptors are a superfamily of transcription factors—proteins that regulate the expression of genes. This superfamily includes receptors for steroid hormones, thyroid hormones, and fat-soluble molecules, such as Vitamins A and D. Nuclear receptors regulate the expression of many genes involved in a broad range of metabolic, reproductive, developmental, and immune response programs. NURSA has a particular focus on metabolism and the development of a number of metabolic disorders, including type 2 diabetes, obesity, lipid dysregulation, and others, as well as on processes of aging and hormone-dependent cancers. Researchers in the NURSA consortium have recently made key discoveries that are increasing knowledge about the role of nuclear receptors in physiology and underlying mechanisms of disease. The long-term goal of NURSA is to translate fundamental observations on the role of nuclear receptors in metabolism into applications that can be used in interventions to treat or prevent disease.

Nuclear Receptor Expression Network Illuminates Control of Metabolism and Other Physiologic Processes: Studying the function of a single nuclear receptor in a single tissue yields information about the receptor's biological function. However, just like looking at only one piece of a puzzle, it does not always give the overall picture with respect to how the receptor may be working with others to govern higher-order biological processes. To begin to address this more complex question, NURSA researchers used a systems biology approach to examine the expression of all 49 mouse nuclear receptors in 39 mouse tissues. When the data were clustered for receptor expression by tissue, the researchers uncovered a hierarchical, integrated transcriptional network tying nuclear receptor function to physiology. At the top of this hierarchy, the researchers unexpectedly observed that the network branched into two major clusters of receptors involved in: (1) reproduction, and (2) nutrient metabolism. Although individual receptor function is important, these results suggest the existence of a higher-order transcriptional network, which extends beyond individual tissues and governs physiology of the entire organism. These experiments examined nuclear receptor expression at a single point in time. In a related study, NURSA researchers measured expression at different time points during the day and night in certain key metabolic tissues. They discovered that 25 of the nuclear receptors were expressed in a rhythmic cycle (i.e., their expression changed at different times during the day and night) in these metabolically-active tissues. While it is known that organisms have a circadian clock that dictates different behaviors at different times, the workings of this clock are unknown. The new data suggest that the nuclear receptors are a link between the circadian clock and metabolism. Together, these studies provide tools for additional research on the biological role of both individual nuclear receptors and nuclear receptors as a superfamily.

Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, and Mangelsdorf DJ: Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell 126: 789-799, 2006.

Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, and Evans RM: Nuclear receptor expression links the circadian clock to metabolism. Cell 126: 801-810, 2006.

Key Role for Nuclear Receptors in Liver Regeneration from Bile Acid Signals: The liver is one of few internal organs that is capable of regenerating itself if part of it is damaged or removed. Up until now, the mechanism by which the body "senses" liver size and the elements that initiate liver regeneration have been elusive. Now, recent studies using mice that had undergone partial liver removal ("partial hepatectomy") have found that an

important role in liver growth and regeneration is played by bile acids. These factors are produced in the liver, stored in the gallbladder, and released into the small intestine to aid in the digestion of fats, after which they are returned to the liver where they may be reused. Bile acids also exert biological effects on liver cells through signaling pathways activated by the nuclear receptor FXR. Researchers hypothesized that, when part of the liver is removed or damaged, remaining liver cells are exposed to relatively higher levels of bile acids, because there are fewer cells to process the same levels of bile acids returning from the intestine. Furthermore, they wondered whether this might be a signal to the body of diminished functional capacity and trigger proliferation of the remaining cells through the FXR pathway. Starting with experiments in mice with intact livers given dietary bile acids, researchers showed that bile acids have a growth-promoting effect on the liver. Then, in partially hepatectomized mice, they demonstrated that bile acids are needed for growth processes during liver regeneration. This effect required bile acid signaling through FXR, because mice lacking this nuclear receptor showed reduced liver regeneration. This set of experiments identifies bile acids as key players responsible for signaling the regulation of liver growth and regeneration, and positions FXR as a key mediator of these processes. It also suggests a dynamic regulatory mechanism influencing liver size based on bile acid flux and functional liver capacity.

Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, Dong B, Huang X, and Moore DD: Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science 312: 233-236, 2006.

RNA INTERFERENCE (RNAi) APPROACHES UNCOVER FUNDAMENTAL PROCESSES

NIDDK-funded investigators are building upon a scientific discovery that was recognized by the award of the 2006 Nobel Prize in Physiology or Medicine to two long-time NIH grantees, Andrew Z. Fire, Ph.D., of the Stanford University School of Medicine and Craig C. Mello, Ph.D., of the University of Massachusetts Medical School. The two researchers were honored for their discovery of RNAi, a mechanism for silencing genes that could lead to new disease treatments. In a seminal laboratory study, Drs. Fire and Mello showed that double stranded RNA can interfere with a gene's activity. RNAi is a technique in which small double stranded RNA, complementary to the mRNA of a given gene, is added to cells. The cells respond by degrading the mRNA, thereby preventing it from being translated into a protein. The technique has proved to be an excellent general method for figuring out the function of genes. Furthermore, RNAi offers the potential to design a new generation of drugs for human diseases that act by inhibiting the expression of certain genes. Both of the following advances use the RNAi approach to glean new understanding into biological processes.

Gene Expression Triggered by Protein Damage Protects Cells from Stress:

Researchers have gained new insights into the processes by which cells respond to environmental stress. Specifically, they examined the stress caused by high salt in the environment (also called hypertonic stress). Hypertonic stress harms cells by causing cellular water loss, cell shrinkage, and protein damage (such as protein unfolding). In a previous study, NIDDK-supported researchers showed that the roundworm, *Caenorhabditis elegans*, adapts to hypertonic stress by increasing expression of the glycerol 3-phosphate dehydrogenase gene. The result is an increase in the small molecule glycerol. More recently, the investigators provided evidence that the increase in glycerol production is essential and specific for *C. elegans* survival in hypertonic conditions. They further sought to identify key signaling pathways and gene expression associated with these conditions. Using RNAi, the researchers determined that a subset of genes is expressed during hypertonic conditions. Many of the genes in this subset code for proteins that function normally to regulate translation and protein folding and to prevent the accumulation of damaged

or denatured proteins in the cell cytoplasm. Thus, the investigators suggest that protein damage may be the signal that triggers the hypertonic stress response in *C. elegans*. Because misfolded proteins accumulate in various diseases, understanding the underlying molecular mechanisms of protein damage caused by hypertonic stress could provide new insights into other diseases having a protein damage component.

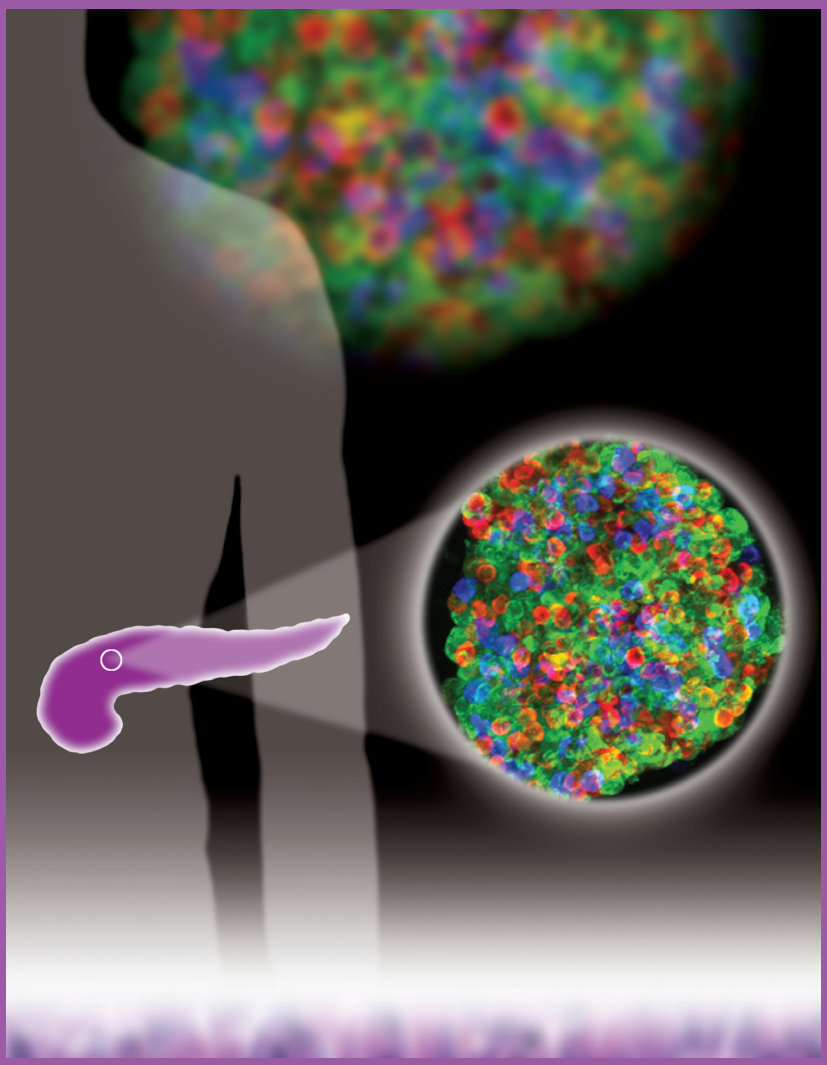
Lamitina T, Huang CG, and Strange K: Genome-wide RNAi screening identifies protein damage as a regulator of osmoprotective gene expression. Proc Natl Acad Sci USA 103: 12173-12178, 2006.

The Effects of Aging on "Proteotoxicity":

NIDDK-supported research is shedding light on a poorly understood aspect of the aging process: protein aggregation. As humans age, deposits of an aggregated protein fragment ($A\beta_{1-42}$) accumulate in the brain. In autopsies of people with Alzheimer's disease, these aggregates have been found to be larger and more numerous than in the brains of other individuals, although the link between the aggregates and disease symptoms remains a subject of scientific debate. Researchers have noted an important connection between the aging process and an important signaling pathway (the insulin/insulin-like growth factor-1 signaling pathway). When functioning normally, this pathway acts to reduce expression of a group of proteins called "chaperones," which have the job of preventing protein aggregation.

This observation has led researchers to hypothesize that protein aggregation leads to senescence in normal worms. RNAi used to interrupt this signaling pathway results in higher chaperone levels, and longer life. The aggregated protein fragment is not found naturally in worms, but when it is placed in them experimentally, it accumulates in aggregates as the worms age. Researchers recently found that RNAi interruption of the signaling pathway associated with aging in worms that express the aggregated protein fragment not only made them live longer, but it also substantially delayed and reduced formation of these aggregates. Their results showed that worms fared worse when they had large numbers of smaller aggregates than when they had smaller numbers of larger clumps. This finding suggests that the smaller aggregates are the more toxic ones. The researchers found that chaperones controlled by the signaling pathway detoxify the aggregates not only by eliminating them, but also by clumping smaller, more toxic aggregates into larger, less toxic forms. Similar processes may account for the importance of this signaling pathway in human aging, and it may be possible to modulate chaperones to prevent or delay some of the burdensome effects of growing older.

Cohen E, Bieschke J, Perciavalle RM, Kelly JW, and Dillin A: Opposing activities protect against age-onset proteotoxicity. Science 313: 1604-1610, 2006.



The ability to see insulin-producing beta cells within the body may prove invaluable in terms of diagnosing and managing diabetes, and could help researchers better understand the life cycle of these cells and how they are damaged in this disease. The problem is complex, because beta cells are one of several cell types in the islet, and represent only a small fraction of the total cells in the pancreas. In this image of a human islet, the insulin-producing cells are stained green, whereas two other cell types are stained blue or red. (Other colors result when these three basic colors overlap in the three-dimensional image.) A recent NIDDK-sponsored research advance toward imaging beta cells is described in this chapter.

Islet image courtesy of Drs. Marcela Brissova and Alvin Powers, Vanderbilt University.

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 launches a misguided attack and destroys the beta cells of the pancreas. These beta cells, which are found within tiny cell clusters called islets, produce the hormone 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 about 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 adults 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 orally administered medications; some patients also need to take insulin. There are also an estimated 54 million adults in the U.S. who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes. 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 glucose level 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.

Genetic Mutation Linked to Some Forms of Neonatal Diabetes Mellitus: Novel mutations in a component of an ion channel in insulin-producing beta cells have been found to contribute to the development of neonatal diabetes. This form of diabetes appears in the first months of life, and may either be permanent, or transient—with the possibility of relapse later in life. Researchers studying families with neonatal diabetes screened their DNA for mutations in a gene (*ABCC8*), which encodes one subunit of a transmembrane ion channel in the pancreatic beta cells. Mutations in the gene for the other subunit of this channel (*KCNJ11*) have also been shown to cause neonatal diabetes. Investigators found mutations in the *ABCC8* gene in 9 of 34 patients with neonatal diabetes—in whom no other genetic defect had been previously identified. The mutations are thought to upset the careful regulation of potassium and calcium ions inside and outside of the beta cells. Disrupting this balance inhibits the release of insulin from the beta cells, leading to a dangerous rise in overall blood glucose levels. Fortunately, patients with mutations in either *ABCC8* or *KCNJ11* responded favorably to treatment with a class of orally-administered drugs known as sulfonylureas, simplifying their treatment. These drugs act by binding to the complex of proteins that make up the ion channel, increasing their surface expression, and inhibiting the release of potassium ions from the beta cell. These actions allow insulin release in response to elevated blood glucose. These studies identify a novel mechanism for the development of a significant fraction of permanent and transient neonatal diabetes in a particularly vulnerable group of individuals, and shed light on the biochemical mechanism of action of the drugs used to treat it.

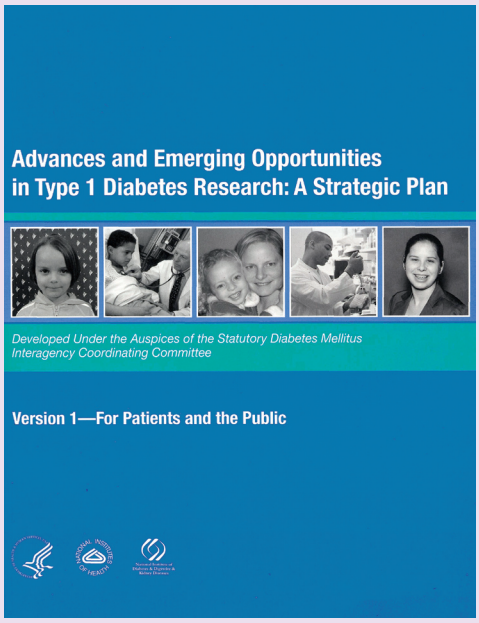
Babenko AP, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, and Froguel P: Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med 355: 456-466, 2006.

Yan FF, Casey J, and Shyng SL: Sulfonylureas correct trafficking defects of disease-causing ATP-sensitive potassium channels by binding to the channel complex. J Biol Chem 281: 33403-33413, 2006.

Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan

Type 1 diabetes is a devastating disease that results when the immune system destroys the insulin-producing beta cells of the pancreas. Individuals with type 1 diabetes must check their blood glucose levels many times a day with finger sticks, carefully monitor their food intake and physical activity, and administer insulin with injections or a pump. Type 1 diabetes can cause life-threatening complications, such as heart disease, blindness, and kidney failure. Continued research is critically important to reduce the toll of type 1 diabetes and its complications on human health.

To accelerate research progress, a Type 1 Diabetes Research Strategic Plan has been developed by the statutory Diabetes Mellitus Interagency Coordinating Committee, chaired by the NIDDK, with broad input from external scientific and lay experts and patient advocacy groups. The Strategic Plan is expected to serve as a scientific guidepost to inform future type 1 diabetes research efforts and propel research progress on the understanding, prevention, treatment, and cure of type 1 diabetes and its complications. In the news release announcing the Plan's completion, the NIH Director, Elias A. Zerhouni, M.D., noted that: "Research has greatly improved the length and quality of life of people with type 1 diabetes, and it has lowered the risk of developing certain serious complications, such as retinopathy and kidney failure. However, many challenges remain in combating this complex autoimmune disease. The Strategic Plan sets forth a cogent, multifaceted approach to future research that soundly addresses these challenges." The Plan is focused around six overarching goals of type 1 diabetes research: identify the genetic and environmental causes; prevent or reverse the disease; develop cell replacement therapy; prevent or reduce hypoglycemia; prevent or reduce complications; and attract new talent and apply new technologies to research. Based on the same general content, two versions of the Plan were developed for: (1) patients and the public, and (2) the scientific research community. The Strategic Plan was released in August 2006, and can be accessed on the NIDDK's website at: *www.T1Diabetes.nih.gov/plan*



Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan, released in August 2006, describes a comprehensive, long-range approach to guiding type 1 diabetes research toward improved care, prevention, and cure for this devastating disease. Two distributed versions—one for patients and their families, and one for the scientific community—differ primarily in level of detail. Both were developed with broad input from stakeholders, including the scientific community, patients, and patient advocacy groups.

"Seeing" New Progress in Type 1 Diabetes—Imaging Beta Cells: The goal of visualizing insulin-producing beta cells in the pancreas is closer to realization than ever before. Why is it important to "see" beta cells? Type 1 diabetes is usually diagnosed late in disease progression, when most of the beta cells have been destroyed by an autoimmune attack. Currently, there is no way to detect the first signs of beta cell destruction or to monitor beta cell loss as the disease progresses. The ability to visualize beta cells could enable earlier intervention to stop or slow disease progression, as well as permit scientists to monitor response to therapy. Toward this goal, researchers used imaging technology (positron emission tomography or PET) to visualize beta cells *in vivo* in a rat model of type 1 diabetes. They used a labeled form of a compound (DTBZ), which binds to a protein found in some cells of the body, including beta cells. Thus, when the labeled compound bound to the beta cell protein, the beta cells could be visualized by sophisticated imaging. As the beta cells were destroyed during progression to type 1 diabetes, the researchers observed a decrease in the uptake of the labeled compound in the pancreas that foreshadowed the loss of blood glucose control. This approach permitted them to noninvasively monitor beta cell destruction as the laboratory animals transitioned from a healthy to a disease state. This labeled compound is already used in humans for clinical imaging of the brain. Therefore, this approach has high potential to be translated to humans and could be enormously helpful in monitoring disease progression and response to therapy in type 1 diabetes.

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Type 2 Diabetes and Obesity—the RBP4 Protein and Implications for Medical Care: A potential new diagnostic marker and therapeutic target is emerging for type 2 diabetes, a devastating and increasingly prevalent disease. Building on their earlier research in mice, scientists tested levels of a protein called retinol binding protein 4 (RBP4) in blood samples from patients with type 2 diabetes or people with such risk factors as obesity, a family history of the disease, or levels of blood glucose that were elevated but not yet diabetic. The high blood glucose levels seen in type 2 diabetes reflect the impaired response, or "resistance," of multiple body tissues to the hormone insulin, and the eventual inability of the body to produce sufficient insulin to overcome this resistance. Levels of the RBP4 protein correlated with insulin resistance in people who were obese, either with or without type 2 diabetes. The scientists also studied another group of individuals who underwent exercise training after having been newly diagnosed with type 2 diabetes or abnormal glucose levels. In this group, those whose body's responsiveness to insulin improved the most from the exercise also had the greatest change in their RBP4 levels. Interestingly, RBP4 levels also correlated with measures of insulin resistance in people who were not obese or diabetic, but who had a close relative with type 2 diabetes, and thus a potential genetic predisposition to the disease. Additionally, the researchers found that elevated RBP4 levels occurred together with cardiovascular risk factors often associated with insulin resistance, such as high triglyceride levels and blood pressure, and abdominal obesity. The results of these studies not only suggest that a blood test for RBP4 levels may be a convenient way to assess risk for type 2 diabetes, but also illuminate a potential new strategy for combating this disease, namely, the development of drugs that could lower RBP4 levels. (For more information about RBP4, please see the Scientific Presentation by Dr. Barbara Kahn in the Obesity section.)

Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, and Kahn BB: Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 354: 2552-2563, 2006.

Unraveling the Complexities of Childhood Diabetes: Previously known primarily as a disease of adults, type 2 diabetes is now increasingly observed in children, particularly minority youth. Little is known about the prevalence of risk factors for diabetes or cardiovascular disease (CVD) in children and youth; however, recent research has begun to shed some light on this issue. Researchers who are conducting a pilot study for a larger prevention trial (called HEALTHY) studied over 1,700 eighth grade students in 12 U.S. middle schools with predominantly minority students. They found a high prevalence of three major risk factors for diabetes—impaired fasting glucose (i.e., pre-diabetes), high levels of fasting insulin (suggestive of insulin resistance), and overweight. A surprisingly high 15 percent of the students had all three risk factors. The researchers also observed significant differences across racial and ethnic groups; for example, American Indians had the highest prevalence of overweight. Overall, nearly half of the students were overweight or at risk for overweight. Many children also had CVD risk factors, such as high blood pressure and elevated lipid levels, which were associated with overweight.

The data collected during the pilot study suggested that middle schools are appropriate targets for population-based efforts to decrease risk for overweight and diabetes. In August 2006, the NIDDK launched the full-scale HEALTHY trial, which is directed at reducing risk factors for type 2 diabetes in middle-school age children. Half of the 42 enrolled schools are receiving the intervention, which consists of: environmental changes to school food service and physical education class activities; behavior change activities; and communications and promotional campaigns. Identifying new strategies to prevent risk factors for diabetes is extremely important because recent data estimate that 1 in 14 children in the U.S. between 12 and 19 years of age have pre-diabetes—and many of the children with pre-diabetes have CVD risk factors.

What about children who already have type 1 or type 2 diabetes, who are not addressed by the HEALTHY pilot study? New insights are beginning to emerge from the Search for Diabetes in Youth (SEARCH) study, which is identifying cases of diabetes in children and youth under 20 years of age in six geographically dispersed populations encompassing the ethnic diversity of the U.S. SEARCH researchers have estimated that 1 of every 523 youths had physician-diagnosed diabetes in 2001, making diabetes one of the leading chronic diseases in children and adolescents. SEARCH researchers have also demonstrated that the prevalence of multiple CVD risk factors is high in children and adolescents with diabetes. CVD risk factors were present in youth with both type 1 and type 2 diabetes, but were more common in adolescents with type 2 diabetes. SEARCH has also demonstrated that about nine percent of adolescents with diabetes have moderate or severely depressed mood. This is similar to findings in youth without diabetes, but importantly, in young diabetes patients depressed mood was associated with poor diabetes control and a higher likelihood of emergency room visits. Another important observation was that higher body mass index (a measure of weight relative to height) was associated with younger age at diagnosis of type 1 diabetes, but only in children with substantially reduced beta cell function. These data suggest that, only among individuals with already compromised beta cell function and/or high rate of beta cell loss, overweight accelerates type 1 diabetes onset. The same study found that lower birth weight was associated with earlier age at onset of type 1 diabetes. These key observations are helping to inform treatment strategies for children with diabetes. As data continue to emerge, the knowledge gained from these studies will contribute to identifying the best ways to diagnose, treat, and manage diabetes in children, and ultimately help to reverse the trend of increasing rates of type 2 diabetes in this population.

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Jago R, Harrell JS, McMurray RG, Edelstein S, El Ghormli L, and Bassin S: Prevalence of abnormal lipid and blood pressure values among an ethnically diverse population of eighth-grade adolescents and screening implications. Pediatrics 117: 2065-2073, 2006.

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The SEARCH for Diabetes in Youth Study Group. The burden of diabetes mellitus among US youth: prevalence estimates from the SEARCH for Diabetes in Youth study. Pediatrics 118: 1510-1518, 2006.

Williams DE, Cadwell BL, Cheng YJ, Cowie CC, Gregg EW, Geiss LS, Engelgau MM, Narayan KM, and Imperatore G: Prevalence of impaired fasting glucose and its relationship with cardiovascular disease risk factors in US adolescents, 1999-2000. Pediatrics 116: 1122-1126, 2005.

Genetic Variant Raises Risk for Type 2 Diabetes Development: Researchers have confirmed that a variant in a specific gene (*TCF7L2*) confers susceptibility to type 2 diabetes in individuals who participated in the landmark Diabetes Prevention Program (DPP). The DPP was the first major clinical trial to show that diet and exercise can effectively reduce the risk of developing diabetes in overweight people with pre-diabetes. In their analysis of 3,548 DPP participants, researchers found that 40 percent of the people had one copy of the gene variant, while 10 percent of participants had two copies. When people had two copies, their risk of developing diabetes was about 80 percent higher compared to people without the variant. The DPP population included people from minority groups who are disproportionately affected by type 2 diabetes. Encouragingly, even the DPP participants at highest genetic risk benefited from healthy lifestyle changes as much or perhaps more than those who did not inherit the variant. This finding emphasizes that people at risk for developing type 2 diabetes can benefit greatly by implementing a healthy lifestyle—whether they are overweight, have elevated blood glucose levels, or have the gene variant. The researchers further showed that the gene variant affected only one of the two major hallmarks of type 2 diabetes. It impaired the ability of pancreatic beta cells to secrete insulin, but it did not affect the ability of target tissues to respond to insulin (insulin resistance). Additional research to understand how the gene variant plays a role in disease could provide novel insights into the molecular basis of diabetes and lead to targeted strategies for prevention and therapy.

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STORY OF DISCOVERY

New Technology for Diabetes—Continuous Glucose Monitors

Sometimes, what you don't know *can* hurt you. For people with type 1 diabetes, undetected high or low blood glucose levels can have severe health consequences—including heart disease, blindness, and coma. With advanced technology, however, patients now have the opportunity to monitor their blood glucose (sugar) levels continuously, rather than just a few times a day. Developed with NIDDK, industry, and other support, these new, wearable continuous glucose monitors sound an alarm when glucose levels soar or plunge to dangerous levels—especially important during sleep. They also generate important data, in real time, on trends in glucose levels as they fluctuate throughout the day and night. With this new wealth of knowledge, people with type 1 diabetes may greatly improve their daily disease management by better adjusting the timing and dosages of insulin, the hormone required to prevent excessive high blood glucose, and by eating or taking other action to raise low blood glucose. The monitors also have potential to help some type 2 diabetes patients control their blood glucose levels. Finally, the realization of continuous glucose monitoring technology is a key step toward developing a mechanical replacement for disease-ravaged pancreatic beta cells, the cells that normally monitor glucose levels and produce insulin, but are destroyed in type 1 diabetes.

Decades ago, reliable and practical methods for glucose monitoring were not available, yet control of glucose levels in the body is crucial. Glucose is obtained from food and is also made by the liver. In healthy people, insulin from pancreatic beta cells directs cells throughout the body to absorb glucose from the blood for use as energy. Without beta cells, however, type 1 diabetes patients face daily the arduous tasks of glucose monitoring, insulin administration, rigorous meal planning, and other efforts to control blood glucose. Without sufficient insulin, cells are deprived of energy, and, over time, high blood glucose levels greatly increase risks for heart disease, blindness, kidney failure, nerve damage, and other severe complications. Administering too much insulin, however, can lead to dangerously low glucose levels, or hypoglycemia, which can result in coma or death if untreated, and is especially feared during sleep. Thus, researchers have long sought to develop improved glucose-monitoring methods.

For years, people with type 1 diabetes could only check their glucose levels by testing urine, a method that was not very accurate or useful. In the 1960s, scientists invented the first meter to measure glucose in the blood. By the 1980s, blood glucose meters were widely used, and, with further improvements, remain so today. Important to any developing technology is a way to evaluate it, and in the 1980s, NIDDK-supported scientists devised a method (error grid analysis) to assess the accuracy of glucose-measuring devices in diabetes management.

The tremendous health benefits of intensive blood glucose control were demonstrated in the early 1990s by a landmark, NIDDK-supported clinical trial, the Diabetes Control and Complications Trial (DCCT). This trial, which was possible because of the availability of glucose-monitoring devices, showed that intensive control greatly reduced development of diabetic eye disease, kidney disease, and nerve damage in people with type 1 diabetes, and the

STORY OF DISCOVERY

ongoing follow-up study recently demonstrated reduced risk for heart disease and stroke. The intensive control regimen is difficult, however, because it requires multiple painful finger sticks each day to draw blood for testing, as well as more frequent insulin administration than was the standard practice prior to the DCCT. The DCCT also revealed that intensive control to avoid high blood glucose levels and future complications had a serious trade-off: an increased immediate risk for hypoglycemia. With the difficulties of intensive glucose control and the threat of hypoglycemia, type 1 diabetes patients still rarely achieve recommended glucose levels. The DCCT thus also underscored the critical importance of research to improve methods for blood glucose control.

By the late 1990s, measurement of glucose in the blood had proven useful for several checks per day, but it was not readily amenable to continuous monitoring. By way of analogy, patients can see a few "snapshots" of their glucose levels per day with blood glucose meters, but miss what happens in between; with continuous monitors, patients would see an entire movie that captured glucose highs, lows, and trends throughout the day and night. Thus, scientists were actively investigating another route to assess glucose that would be both safe and practical for continuous monitoring—the interstitial fluid in tissues under the skin. A critical research question was whether glucose levels measured by a sensor in the interstitial fluid would reflect glucose levels in the blood. The answer was "yes," as shown in studies in animals and humans, by several research groups supported by the NIDDK and industry. A continuous monitor was first approved by the FDA in 1999. The glucose values obtained from this device were not as accurate as direct blood glucose measures, and could only be assessed retrospectively, not in real time. But the continuous monitor could amass hundreds of glucose readings per day for subsequent analysis by health care providers and patients.

Further research culminated in the FDA approval, in 2006, of new continuous glucose monitors for people with diabetes. These monitors provide glucose readings in real time, every 5 minutes; display trend data so patients know whether their glucose levels are rising or falling; and sound alarms when levels are too high or low—including at night, during sleep. Before taking action to adjust high or low glucose, patients will still need to confirm readings from these new monitors with a traditional finger stick and blood glucose meter. Additionally, the scientists found that, when blood glucose levels change, there is a time lag of a few minutes before a change is detected in the interstitial fluid. Patients will thus need to consider this small lag time in daily disease management. Importantly, however, researchers have demonstrated that, with the use of continuous glucose monitoring devices, patients spend less time in high and low blood glucose ranges, and more time in the recommended range. This achievement resulted from research investments by both NIDDK and industry.

Continuous glucose monitoring technology will enable people with diabetes and their doctors to better predict how meals and daily activities will affect blood glucose, and to adjust and personalize their disease management accordingly to help preempt long-term diabetic complications and acute episodes of hypoglycemia. An ongoing and critical area of NIH-funded research is the evaluation of these monitors for use in children, because they are currently approved only for adults. Finally, a key feature of one of the approved

monitors is that it transmits its data to an insulin pump. Insulin pumps are small, pager-sized machines that can deliver insulin to patients continuously in a small basal amount and provide larger boluses when needed, for example at mealtime. Although patients still must be actively involved in determining their insulin doses, based on glucose readings and other factors, this first pairing of a continuous monitor and pump has major implications. With NIDDK funding, scientists are now developing algorithms that will one day "close the loop" between the glucose monitor and insulin pump, creating an "artificial pancreas" to automate insulin delivery in response to the body's needs. In September 2006, the NIDDK Acting Director, Dr. Griffin Rodgers, presented testimony on artificial pancreas development efforts before the Senate Homeland Security and Governmental Affairs Committee. This testimony is available on the NIDDK Website (*http://www.niddk.nih.gov/federal/planning/Rodgers092706ArtificialPancreasTestimonyHSGAC.pdf*). Even before an artificial pancreas is developed, patients can improve their health now, with the unprecedented knowledge gained from continuous glucose monitors.

National Diabetes Education Program: Type 2 Diabetes Often Goes Unnoticed

One-third of adults with diabetes do not know they have it. Scientists from the NIH and the Centers for Disease Control and Prevention (CDC) analyzed data on U.S. adults aged 20 years and older from the CDC's National Health and Nutrition Examination Survey (NHANES) during two time periods: 1988 to 1994 and 1999 to 2002. Comparison of data from these two time periods revealed that the prevalence of diagnosed diabetes rose from about 5.1 percent to 6.5 percent. However, the percentage of adults with undiagnosed diabetes did not change significantly. About 2.8 percent of U.S. adults—or one-third of those with diabetes—still do not know they have it. The study noted that type 2 diabetes accounts for about 95 percent of all diabetes cases and virtually all undiagnosed diabetes cases.

The study also found that another 26 percent of adults have a form of pre-diabetes, a condition in which a person's blood glucose is high but not yet diagnostic of diabetes. Pre-diabetes usually causes no symptoms. However, many people with the condition develop type 2 diabetes within the next 10 years. Also, pre-diabetes substantially raises the risk of a heart attack or stroke even if type 2 diabetes does not develop.

Who is at risk? Risk factors for developing pre-diabetes and type 2 diabetes include being age 45 or over, having a family history of diabetes, being overweight, or having a history of gestational diabetes during pregnancy. Certain ethnic populations are also at high risk (e.g., African American, Hispanic/Latino American, American Indian and Alaska Native, Asian American, and Pacific Islander). For more information on type 2 diabetes and its risk factors, please see: *http://diabetes.niddk.nih.gov/*

The good news is that NIDDK-supported research has shown that people with pre-diabetes can prevent or delay the development of type 2 diabetes by losing a modest amount of weight by reducing the number of calories in their diet and increasing physical activity. The National Diabetes Education Program (NDEP), sponsored by the NIH, CDC, and over 200 partner organizations, is disseminating this important message to people at risk for type 2 diabetes with the *Small Steps. Big Rewards. Prevent Type 2 Diabetes.* campaign. The NDEP also spearheads the *Control Your Diabetes for Life* campaign, which encourages people with diabetes to control their blood glucose levels, as well as their blood pressure and cholesterol levels. Information about these campaigns and other diabetes education programs can be found at: *www.ndep.nih.gov/*

Diabetes Prevention Program: Continuing Benefits from Research

Type 2 diabetes is a devastating and chronic disease. But, as the NIDDK-led Diabetes Prevention Program (DPP) clinical trial demonstrated, those at high risk for this disease can prevent or delay its onset. In the years since this landmark result was reported, the Institute has brought its important prevention message to health care professionals and the public through the educational campaign: *Small Steps. Big Rewards. Prevent Type 2 Diabetes*. The NIDDK is also vigorously supporting further research on type 2 diabetes to improve people's lives.

TYPE 2 DIABETES AND THE DPP CLINICAL TRIAL

Type 2 diabetes increases risk for cardiovascular disease, kidney failure, blindness, and other debilitating conditions. It can strike anyone, but disproportionately affects certain minority groups, including African Americans, Hispanic Americans, American Indians, Alaska Natives, and Native Hawaiians. Risk increases with a family history of the disease and with older age, but, alarmingly, type 2 diabetes is increasingly being seen in children. Overweight and obesity are major risk factors for type 2 diabetes and are likely driving the rise in this disease in children. Gestational diabetes, or diabetes diagnosed during pregnancy, occurs in about seven percent of U.S. pregnancies, and puts both mother and child at heightened risk for type 2 diabetes for the rest of their lives.

The DPP clinical trial was conducted at 27 centers throughout the U.S. The volunteers were adults at high risk for type 2 diabetes: they had "pre-diabetes"—blood glucose levels higher than normal but not yet diabetic—and they were also overweight. They were randomly assigned to different groups. The "lifestyle intervention" group received an intensive program to reduce their body weight by a relatively modest 5 to 7 percent, through moderate exercise and reducing dietary fat and calories. The two other groups were given standard lifestyle recommendations along with either the diabetes drug metformin or a placebo. The DPP demonstrated that the lifestyle intervention reduced risk for type 2 diabetes by a dramatic 58 percent. The metformin intervention reduced risk by 31 percent. These interventions worked in all ethnic and racial minorities studied and in men and women, including women with a history of gestational diabetes. Participants over 60 years of age responded particularly well to the lifestyle intervention, whereas both metformin and the lifestyle intervention were similarly effective for the younger participants (ages 25 to 44) and for participants who were very obese.

At the conclusion of the DPP trial, all of the volunteers were given the opportunity to participate in the lifestyle sessions that the intensive lifestyle intervention group had received because this intervention was proven to be so successful.

SMALL STEPS. BIG REWARDS. PREVENT TYPE 2 DIABETES.

This multicultural educational campaign, based on the results of the DPP, brings the message that modest weight loss through dietary change and moderate exercise (small steps) can prevent or delay type 2 diabetes (big rewards). It was developed by the National Diabetes Education Program (NDEP), which is jointly sponsored by the NIDDK and the Centers for Disease Control and Prevention and involves many partner organizations. Campaign materials are tailored for different audiences, including older adults and minority groups at high risk for type 2 diabetes. In 2006, the NDEP launched a new component of this campaign, a prevention message focused on women with a history of gestational diabetes (GDM) and their children.

FURTHER RESEARCH PROGRESS

Now, over 5 years since the report of the Diabetes Prevention Program's key finding—that type 2 diabetes prevention is possible—the DPP continues to provide valuable results. As one example, scientists recently found that the lifestyle intervention reduced the occurrence of urinary incontinence in women with pre-diabetes. In another study, scientists found that a gene variant confers susceptibility to type 2 diabetes in the DPP participants, and that the lifestyle intervention greatly reduced risk for diabetes even in those who had this genetic variation. This study thus builds on a previous report associating this gene with type 2 diabetes—an important result given the complexity of diabetes genetics—and emphasizes that, with healthy lifestyle changes, genetics is not necessarily destiny.

Building on the success of the DPP, the NIDDK is now supporting a follow-up study of the DPP participants, the DPP Outcomes Study (DPPOS). The DPPOS is assessing the durability of the effect of the interventions on the development of diabetes and on maintenance of weight loss, and whether the interventions impact the development of cardiovascular disease. The NIDDK is also encouraging demonstration and dissemination research projects to explore various strategies for translating the DPP results from the clinical trial setting to communities.

The DPP and DPPOS are part of the NIDDK's broad spectrum of basic, clinical, and translational research on type 2 diabetes. This research is strengthening our understanding of this disease and accelerating the development and testing of prevention and treatment strategies.

RESEARCH VOLUNTEERS – ADVANCING PROGRESS AND PROVIDING HOPE FOR MILLIONS OF AMERICANS

The success of the DPP simply could not have happened without the over 3,000 volunteers who participated in this clinical trial. On the following pages, two of the DPP participants share their stories of why they volunteered for this trial, and how they continue to prevent type 2 diabetes.

PATIENT PROFILE

Carol Baker

Gestational Diabetes and the Diabetes Prevention Program

Carol Baker always knew she was at high risk for diabetes. All four of her grandparents, as well as her mother and father, had developed the type 2 form of the disease. "Throughout my entire life my parents kept telling me that I was going to get diabetes. They told me 'be careful…watch out for it,'" says the now 45-year-old substitute teacher and mother of five daughters.

But the disease manifested itself in a way Carol had not anticipated. During her second pregnancy, at age 27, she was diagnosed with gestational diabetes mellitus (GDM), and received the same diagnosis in each pregnancy, thereafter.

GESTATIONAL DIABETES MELLITUS, OR GDM

GDM is a form of diabetes that occurs during pregnancy. Like other forms of diabetes, it's characterized by high blood glucose levels and can have serious consequences. Left untreated or uncontrolled, gestational diabetes can result in babies being born very large and with extra fat, which can make delivery difficult and more dangerous for both mother and child. It also can lead to babies with breathing problems and low blood glucose right after birth.

For most women, gestational diabetes goes away after the baby is born, but leaves them at greater risk for the disease during subsequent pregnancies. Gestational diabetes also leaves both mother and child at increased risk for type 2 diabetes for the rest of their lives.

Carol Baker (on left) with her family (from second left to right): Allie, Jennifer, Molly, Katie, Natalie, and Bryce.

Despite her family history of type 2 diabetes, Carol remains free of this devastating and chronic disease—18 years after her first GDM diagnosis. "I firmly believe the reason I do not have type 2 diabetes is because of the Diabetes Prevention Program (DPP) I participated in," says Carol.

TAKING CONTROL

Carol's five daughters range in age from 12 to 22. Allie, her oldest daughter, was adopted by Carol and her husband. Carol had no problems during her first pregnancy, with daughter Katie, now 20. But she had gestational diabetes during each of her next three pregnancies, with Natalie, 18, Jenne, 15, and Molly, 12. "All of my babies were very large, each over nine pounds." says Carol. "But fortunately," she adds, "everything else was normal." Having always known of her risk for type 2 diabetes, in the mid 1990s, Carol responded to an ad in the health section of a local newspaper asking for volunteers to take part in something called the Diabetes Prevention Program, or DPP.

PATIENT PROFILE

BENEFITS OF PREVENTION

The DPP volunteers were randomly assigned to one of three groups, to test whether different interventions could prevent type 2 diabetes in people at high risk, including those from minority populations and others who had excess weight and blood glucose levels that were higher than normal, but not yet diabetic. Carol's group received a drug called metformin along with standard lifestyle recommendations. Another group received the standard lifestyle recommendations and a placebo. The third group received a more intensive lifestyle intervention program aimed at modest weight loss through improved diet and moderate exercise. The DPP trial showed that this lifestyle intervention dramatically reduced risk for type 2 diabetes. The metformin intervention also reduced risk, although not by as much overall.

The DPP study ended in 2001, but Carol remains vigilant. "I'm very successful at eating properly and taking my medication," says Carol. "I watch my portion sizes, eat whole grains, lean meats, and fruits and vegetables, and I encourage my daughters and husband to do the same." She also strives to keep exercising. "I know I should try to get 30 minutes of physical activity, 5 days a week. Some months I do better than others." Her exercise usually entails walking outdoors or on her treadmill at home, or working out in the basement gym of a friend. She also gets her blood glucose checked every 6 months. "I'm very faithful about these check-ups," she says. "I never miss them."

Fortunately, Carol says, "I have always encouraged my children to lead healthy lifestyles because of their family history of diabetes." And it has paid off—even for the daughters from pregnancies affected by gestational diabetes. These daughters are not overweight, "and so far, no diabetes," she adds, thankfully.

Based on results of the DPP, the National Diabetes Education Program (NDEP), led by the NIDDK and the Centers for Disease Control and Prevention, along with many partner organizations, developed the educational campaign: *Small Steps. Big Rewards. Prevent Type 2 Diabetes*. Recently, the campaign added a new prevention message: "*It's Never Too Early To Prevent Diabetes. A Lifetime of Small Steps for a Healthy Family*." This message raises awareness that women with a history of gestational diabetes and their children have an increased risk for type 2 diabetes. It also emphasizes that lifestyle changes can reduce this risk.

"I have always encouraged my children to lead healthy lifestyles because of their family history of diabetes." And it has paid off—even for the daughters from pregnancies affected by gestational diabetes. These daughters are not overweight, "and so far, no diabetes," says Carol.

Carol Baker understands this very well. In April 2006, at the launch of this new prevention message, Carol was invited to speak, and her words were loud and clear: "I am proof that diabetes prevention is proven, possible and powerful, and I am going to continue taking these 'Small Steps' in an effort to prevent type 2 diabetes from affecting my life or the lives of my children."

NIDDK RESEARCH

The NIDDK's extensive portfolio of research on diabetes and its risk factors includes research related to pregnancy, to benefit mother and child. For example, an NIDDK-led initiative has fostered new studies addressing maternal diabetes or obesity during pregnancy and the long-term metabolic effects on the offspring. These studies complement the NIDDK's research on aspects of pregnancy that affect a mother's risk for diabetes.

For more information:

"What I need to know about gestational diabetes" – http://diabetes.niddk.nih.gov/dm/pubs/gestational/index.htm

"It's Never Too Early To Prevent Diabetes. A Lifetime of Small Steps for a Healthy Family" – http://www.ndep.nih.gov/campaigns/SmallSteps/SmallSteps_nevertooearly.htm

PATIENT PROFILE

Irish Stovall

Diabetes Prevention Program (DPP) Helps Ward Off Type 2 Diabetes

When a card arrived in her mailbox asking Irish Stovall if she would be interested in participating in something called the Diabetes Prevention Program (DPP), Irish, whose family has a long history of type 2 diabetes, had no hesitation responding, "yes."

Irish's mother had diabetes, as well as five out of 10 of her siblings, at least one of whom died from the disease. Several others have suffered serious complications, including a stroke and heart attack. "That's why I was so interested in taking part in this program," says 74-year-old Irish, whose lively and engaged personality resonates in her voice.

The year was 1998 and the mailing said the program was being sponsored by the NIDDK and others. It also listed many research centers across the country that were involved, including those in her hometown of Washington, D.C., Howard University Hospital and MedStar Research Institute. Irish was selected to participate because, in addition to her family history, tests showed she had high glucose levels, a risk factor for type 2 diabetes. "I didn't have diabetes at that time and, thanks to the study, I still don't," she says.

In the DPP, participants were randomly assigned to different groups, to test whether different interventions could reduce the risk of type 2 diabetes. Irish was in the lifestyle intervention group. This intervention aimed for relatively modest weight loss through reduced caloric intake, eating less fatty foods and exercising moderately. Irish turned out to be the perfect candidate.

Irish Stovall

"I weighed 229 pounds when I went into the program," she says. "Because of the way I changed my eating habits, I'm down to 178 pounds and feel great," she adds as she proudly rattles off her daily dietary routine. "I no longer eat fried foods. Breakfast usually consists of a bowl of oatmeal and orange juice. Around mid-morning, I eat a piece of fruit. I also drink six to eight glasses of water a day, which helps to hydrate me and curbs my appetite. I try to eat a green vegetable every day and no more than three ounces of meat. For snacks I have a salad, fruit or yogurt, and I try not to eat anything after 7:30 at night." As for exercise, Irish says she walks three to four miles outdoors 5 days a week. During inclement weather when she can't get out, she exercises on the treadmill in her basement. She also has a supportive

spouse. "My husband is 77 years old," says Irish. "His mother and sisters had diabetes, but he doesn't. We encourage each other to eat well and exercise. Like me, he exercises every day."

"I weighed 229 pounds when I went into the program," she says. "Because of the way I changed my eating habits, I'm down to 178 pounds and feel great."

The DPP ended in 2001, but Irish is currently taking part in the ongoing DPP Outcomes Study (DPPOS). She says she would advise anyone with an interest in his or her health to take part in studies like these. As part of the DPPOS study, Irish goes twice a year for a "very thorough" checkup. "They take my blood pressure not only from my arm, but my leg, as well. They weigh me, give me an electrocardiogram, check my skin and ask me about my diet and exercise routine. I also have a coordinator who monitors and advises me."

Her message to others: "Try to eat more healthy types of foods—and exercise."

For more information:

"Am I at Risk for Type 2 Diabetes?" – http://diabetes.niddk.nih.gov/dm/pubs/riskfortype2/index.htm

"Small Steps. Big Rewards. Prevent Type 2 Diabetes" – http://www.ndep.nih.gov/campaigns/SmallSteps/SmallSteps_index.htm

PATIENT PROFILE

Berg Family

What It's Like When Two of Your Children Have Type 1 Diabetes

Aiden Berg was a 14-month-old toddler when he was diagnosed with type 1 diabetes. Two years later his older sister, Heather, was diagnosed with the disease at age 10. If you ask their parents, Toni and Rob Berg, what is the most difficult thing about raising a family when more than one child has diabetes, without hesitation, the answer comes back: scheduling!

"I think of myself as a pretty organized person," says 38-year-old Toni, who works as an airline customer service agent, "but with this disease, we have to stay on top of things all the time." Even then, things can go wrong.

About a month after Heather was diagnosed, the Bergs inadvertently mixed up Heather's and Aiden's doses of insulin, which resulted in a "mini crisis," says Rob. "Heather's dosage was way too much for Aiden, so we were up the entire night monitoring him. Now we always double check everything," adds the 39-year-old accountant. The Bergs have a third child, Dillon, age 8, who so far does not show any signs of the disease. "We check Dillon's blood sugar at least once a month," says Toni, "and keep our fingers crossed."

UNDERSTANDING THE GENETIC LINK

About 1 out of 5 people with type 1 diabetes has a close family member with the disease. To help scientists better understand the genetics of diabetes, the Bergs are currently taking part in a study called the Type 1 Diabetes Genetics Consortium (T1DGC). This consortium is designed to gather valuable information from 2,800 families like the Bergs, with at least two siblings who have type 1 diabetes. The study, sponsored by the NIDDK and the Juvenile Diabetes Research Foundation International (JDRF), involves researchers from around the world—Europe, North America, Asia-Pacific, and the United Kingdom.

The Berg children

The T1DGC is expected to provide a better understanding of the genetics of diabetes, which may suggest valuable new avenues for treating the disease. Furthermore, genetic testing may one day permit very early diagnoses, thereby enabling earlier management of the disease.

The T1DGC is different from many other clinical studies in that it does not test a medical intervention, but rather, is designed to gather valuable information. Ultimately, information about the genetic basis of the disease may not only help identify new therapies but also predict which therapy might be best for a particular person. Finding the genes predisposing

to type 1 diabetes will also enable those at risk to be identified early so they can benefit from future research. The study is closely aligned with the Type 1 Diabetes TrialNet, which is investigating the development, prevention, and early treatment of type 1 diabetes. Both the T1DGC and TrialNet are supported by the *Special Statutory Funding Program for Type 1 Diabetes Research.*

DEALING WITH THE NEWS

Toni and Rob were familiar with diabetes long before Aiden and Heather were diagnosed. Toni's mother died at age 56 from complications of type 2 diabetes, which she developed after having been diagnosed with gestational diabetes during her last pregnancy. Rob's mother also has type 2 diabetes, but has avoided its complications so far.

According to the Bergs, before Aiden's diagnosis, he was manifesting many of the symptoms of diabetes. "At 12 months he had lost weight and was drinking lots of water," says Toni. "I said to our family doctor, 'My God, he has diabetes.'" Toni was told that the weight loss was probably because Aiden had started to walk, and thus, was using more energy. As for drinking lots of liquids, it was summertime and the temperature was very hot. Aiden's symptoms persisted, however, including: lethargy, constant irritability, and extreme thirst. "We were told over and over that children Aiden's age don't get diabetes," say the Bergs. Recent reports from physicians at diabetes centers suggest that type 1 diabetes may be occurring in younger children than was previously recognized. This is a problem because it is much harder to control the disease in infants and young children who cannot recognize or respond to episodes of dangerously low blood glucose (hypoglycemia).

Finally, Aiden was given a blood test and was diagnosed with type 1 diabetes. By that time, he was so sick he had to be taken immediately to the hospital where he spent 2 days in the intensive care unit. "It just sank in that this was going to be life-long," says Toni. She adds that she was overcome by it all, especially knowing the history of what her mother and others in her family had gone through because of the disease. However, things didn't end there.

Two years later, Aiden's sister, Heather, was diagnosed with the disease. According to the Bergs, Heather's blood glucose was always a bit higher than the levels of the rest of the family. One day, while at a diabetes health exposition in Seattle, where the family resides, Heather used a blood glucose tester and her reading came out well above the healthy range. The vendor for the product told the Bergs to make sure to have Heather's blood glucose checked by a doctor.
Toni hesitated. "I was in denial that two of my children could have diabetes," she says. Heather insisted on having the test because she would feel more comfortable knowing one way or the other.
Sure enough, Heather's blood glucose number came out high again. "I still didn't want to believe it—until we got the [hemoglobin] A1c test results—which confirmed for me Heather's diagnosis," says Toni.

"I felt overwhelmed," Toni recalls, "but Heather was brave and never shed a tear." "I can handle it," the precocious Heather told her parents. And handle it, she has.

USING AN INSULIN PUMP

One year after Heather was diagnosed she went on an insulin pump. "She wanted to go on the pump the day she was diagnosed, but we decided we should

PATIENT PROFILE

wait a while," says Rob. Heather has taken to the pump well and it has helped a lot in terms of family scheduling. "Heather is an extremely competent child and pretty much takes care of herself," says Rob.

"It's not as bad as I thought it would be," says Heather, who is now 11. "The shots don't hurt much, and because I'm on the pump, I don't have to have so many pokes. Also, Aiden had diabetes before me, so I kind of knew what to expect." Besides, she adds, "the pump is cool because people think it's a cell phone."

NO TYPICAL DAY

The Bergs say that no day is "typical" for their family, but they certainly keep diabetes-related procedures well under control.

Each morning the Bergs check Aiden's and Heather's blood glucose levels and administer insulin according to need. Then, the family goes over what they're going to have for breakfast so they know how many carbohydrates will be taken in; the same for when lunches are made. "There's no such thing as buying lunch at school anymore," says Toni. Most days the Bergs check in with the school or day care center to see how the kids are doing. After school, blood glucose levels are checked again, and Aiden and Heather have a snack—either with or without carbohydrates, depending on what their glucose levels turn out to be.

The Bergs also are big on sports. "There is always one sporting event or another that the kids play in," says Toni. Rob adds that, "We try to keep them active all year round. Whether it's baseball, swimming, soccer, cheerleading, gymnastics, riding their bikes or playing in the backyard pool, it makes a big difference in their (blood glucose) numbers." In the winter months, those numbers are a bit higher because they are not quite as active as in the summer, which, according to Rob, means more of an insulin adjustment.

In the evening, the family has dinner, and blood glucose levels are checked just before bedtime. Depending on how much Aiden's and Heather's blood glucose levels fluctuate on any given day, "Either Rob or I will get up in the middle of the night and check them again," says Toni.

TAKING PART IN RESEARCH STUDIES

Like many families with a high incidence of diabetes, the Bergs are seeking as much information as possible about the disease. They became involved with the T1DGC study when they stopped by the Benaroya Research Institute's booth at the Diabetes Expo in Seattle and were asked if they would like to participate in diabetes research. They jumped at the opportunity.

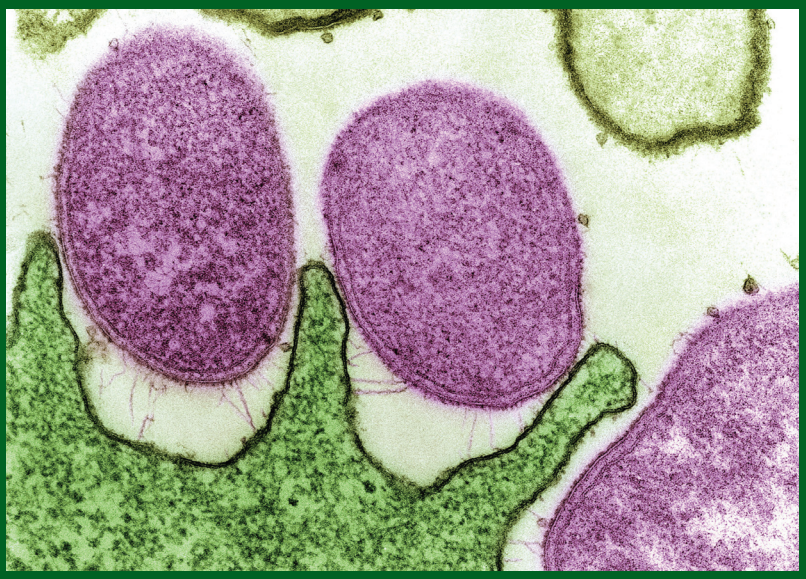
Such studies give hope to families like the Bergs. The T1DGC is expected to provide a better understanding of the genetics of diabetes, which may suggest valuable new avenues for treating the disease. Furthermore, genetic testing may one day permit very early diagnoses, thereby enabling earlier management of the disease. Early intervention could reduce or delay onset of diabetes complications, and

prevent some emergency hospital admissions, such as was necessary for Aiden when he was first diagnosed. Indeed, ongoing research studies are using genetic tests to identify some newborns at high risk for developing diabetes. The studies are indicating that, with careful monitoring of such children, it may be possible to dramatically reduce the likelihood of such hospitalizations.

The hope extends beyond early diagnosis. "Knowing the amount of research going on, we're hopeful that a cure for diabetes will be found by the time our children reach adulthood," says Toni. "We hope and pray other families will participate in this research. The larger the pool of people they have to study, the more they can learn about combating this disease," she adds.

More information on participating in the T1DGC and TrialNet can be found at:

www.t1dgc.org and *www.diabetestrialnet.org*



Colorized electron micrographs of *Helicobacter pylori* bacteria (on top in blue; on bottom in purple) in the human stomach. NIDDK-funded researchers are elucidating important interactions between bacteria and the gastrointestinal tract that contribute to human health and disease. As described in a research advance in this chapter, analysis of the *Helicobacter pylori* genome has shed light on genetic alterations that allow some strains of this common stomach bacterium to become more effective pathogens, causing severe inflammation (gastritis) that can lead to cancer.

Top image: Credit: David McCarthy / Photo Researchers, Inc.

Bottom image: Credit: SPL / Photo Researchers, Inc.

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 non-alcoholic 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 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 experience 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. Gastroparesis is another functional bowel disorder that is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food.

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. Research on controlling intestinal inflammation has potential benefits not only for patients with inflammatory bowel diseases, but also for those at risk of developing colorectal cancer.

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 health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The IL-23 Receptor Is a Major Susceptibility Gene for Inflammatory Bowel Disease:

The two major forms of inflammatory bowel disease (IBD) — Crohn's disease and ulcerative colitis—are thought to arise from a combination of genetic susceptibility and environmental factors. Previous studies have identified mutations in the *NOD2* gene as playing a role in the development of Crohn's disease in humans. Building on this finding, the NIDDK established the IBD Genetics Consortium to speed the search for other susceptibility genes in this complex disease. To identify additional genes, the researchers performed a genome-wide association study by testing more than 300,000 genetic variants in people with and without Crohn's disease. For long stretches, a given DNA sequence may be identical in two different people. However, every so often, one (or more) of the nucleotides varies at a site called a single nucleotide polymorphism or "SNP." The researchers found that, out of the hundreds of thousands of SNPs examined, three were strongly associated with Crohn's disease. Of those, two were in the previously known *NOD2* gene, and the third SNP was in a gene encoding the interleukin-23 (IL-23) receptor. While characterizing the various polymorphisms in the IL-23 receptor gene to determine which ones were the most harmful, the scientists found a polymorphism that appears to protect against development of Crohn's disease. Taken together with previous findings, this important discovery offers new hope for better therapies for patients with this chronic disease.

Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee A, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, and Cho JH: A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 314: 1461-1463, 2006.

"OMICS" TECHNOLOGIES ENABLE UNDERSTANDING OF THE HUMAN DIGESTIVE TRACT AND ITS MICROBIAL COMMUNITY

Research on human health and disease has been greatly enhanced by "omics" technologies. These technologies unlock the vast amounts of information stored in the collection of human genes (genomics);

their products—RNAs expressed (transcriptomics), and proteins translated (proteomics); as well as the many small molecules participating in metabolic processes (metabolomics). These approaches are now being applied with exciting results to understanding the complex functions of the human digestive tract, including studies into the unique regenerative properties of intestinal stem cells and of the microbial world teeming within. Examples follow.

Unique Gene Expression of Adult Gut Stem Cells: Researchers recently used genomic analyses to catalog genes expressed by adult stem cells responsible for renewing the lining of the stomach and small intestine. Because very few stem cells are present in the normal gut, investigators used cells from a mouse model engineered to overproduce them. They found that gastric and intestinal stem cells express a unique set of genes not found in stem cells from other organs. This gene catalogue represents an important tool for investigators to use for elucidating the specialized functions of adult gut stem cells, thereby hastening the time when these cells could be used to treat gastrointestinal diseases.

Importance of the Gut Microbial Community: The human gastrointestinal tract, particularly the large intestine, is home to at least 10 trillion microorganisms. Recent "omics"-based studies have proven extremely helpful for shedding light on the importance of the gut microbial community in human health and disease. Recently, a group of researchers initiated an in-depth study of the collection of microbial genomes (microbiome) in the healthy human intestine. Using stool samples from two healthy adults, they catalogued the genetic diversity of microorganisms in the human large intestine and further defined their distinctive functions within the human body. This effort to catalog gut microbial genes, and the functional attributes associated with the proteins they produce, provides a foundation for future studies into how the gut-microbiome partnership is important for normal human metabolism and intestinal health.

In fact, investigators are already exploring the role of the gut microbiome in human disease using genomic analyses of microbial species from "humanized" animal models and patient samples. For example, researchers have recently used transcriptomic analysis to understand how interactions among specific gut microbial species can affect host energy balance. For these analyses, they used a "humanized" mouse model, raised so that the gut was free of microbes and then colonized with gut microbes commonly found in humans. They found that two microbial species in particular—*Methanobacter smithii* and *Bacteroides thetaiotaomicron*—have a cooperative relationship in digesting fiber that leads to more efficient intestinal nutrient absorption and energy storage as fat. Further research building on this work could lead to new ways to address both obesity and undernourishment.

Researchers have also used genomic analysis to understand digestive disease caused by *Helicobacter pylori* infection, which is extremely common in the U.S. and other countries. While most people develop a mild case of stomach inflammation from the infection, a select few develop more severe, chronic inflammation that can progress to gastric cancer. Upon detailed genomic analysis of bacterial strains isolated from several patients who developed severe gastric disease, results revealed a common gene "signature" among these strains. Also uncovered were unique genetic changes in some strains that allowed them to survive in the stomach as inflammation progressed to gastric cancer. These findings could aid in identifying patients in whom *H. pylori* infection will likely cause severe disease, so that it can be prevented or treated before progressing to gastric cancer.

Giannakis M, Stappenbeck TS, Mills JC, Leip DG, Lovett M, Clifton SW, Ippolito JE, Glasscock JI, Arumugam M, Brent MR, and Gordon JI. Molecular properties of adult mouse gastric and intestinal epithelial progenitors in their niches. J Biol Chem 281: 11292-11300, 2006.

Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, and Nelson KE. Metagenomic analysis of the human distal gut microbiome. Science 312: 1355-1359, 2006.

Oh JD, Kling-Bäckhed H, Giannakis M, Xu J, Fulton RS, Fulton LA, Cordum HS, Wang C, Elliott G, Edwards J, Mardis ER, Engstrand LG, and Gordon JI. The complete genome sequence of a chronic atrophic gastritis Helicobacter pylori strain: Evolution during disease progression. Proc Natl Acad Sci USA 103: 9999-10004, 2006.

Samuel BS and Gordon JI. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. Proc Natl Acad Sci USA 103: 10011-10016, 2006.

LIVER DISEASE RESEARCH

Search for Reasons Underlying Different Hepatitis C Treatment Responses:

Chronic hepatitis C is a major cause of chronic liver disease and the need for liver transplantation in the U.S. Unfortunately, there is a variable response rate to the available standard therapy for chronic hepatitis C—a combination of the drugs peginterferon and ribavirin. African Americans with hepatitis C are less responsive to treatment than Caucasian Americans. To investigate possible reasons for this difference and identify ways to improve treatment regimens, the NIDDK-funded Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) is comparing treatment responses in groups of African Americans and Caucasian Americans. Recent results from this study have more accurately characterized the racial difference in response to treatment. Results showed a significantly lower viral response in African Americans as early as week four of treatment, and a higher rate of "breakthrough," with a return of elevated viral levels later in the course of treatment. The investigators were also able to rule out several potential explanations for the difference in treatment response, including higher viral levels before treatment, sex, age, weight, extent of fibrosis in the liver, and amount of medication taken. Research in this study population is continuing in search of possible virologic, immunologic, and genetic factors underlying the cause of reduced treatment response in African Americans with chronic hepatitis C.

Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucase TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, and Howell CD, for the Virahep-C Study Group. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 131: 470-477, 2006.

Measures of Success in Hepatitis C Treatment:

The standard therapy used to treat chronic hepatitis C consists of a combination of the drugs peginterferon and ribavirin given for approximately 6 months or a year. However, this therapy proves, ultimately, to be ineffective in a large proportion of people with chronic hepatitis C. In an effort to monitor the success or failure of the treatment, current guidelines call for the use of assays to measure hepatitis C virus (HCV) RNA levels during and after treatment as an indicator of lasting response and clearance of the viral infection. Recently, a more sensitive assay of HCV RNA was developed, called the transcription-mediated amplification or "TMA" assay, though its usefulness for monitoring treatment response was unknown. Researchers involved in the NIDDK-supported Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial put the new assay to the test using samples from their study population—patients with chronic hepatitis C who did not respond to prior treatment with interferon. Compared to an older HCV RNA assay, the TMA assay proved more sensitive in detecting HCV RNA and predicting earlier in the course of treatment whether a sustained response was likely. Based on the promising results in this subset of patients, further studies are warranted to determine the usefulness of the TMA assay in monitoring and predicting treatment response in other patients with chronic

hepatitis C. If the results are applicable to other patient groups, the assay could represent a better way to predict non-responsiveness to standard therapy, and also allow for early discontinuation of therapy in patients who would not benefit from further treatment.

Morishima C, Morgan TR, Everhart JE, Wright EC, Shiffman ML, Everson GT, Lindsay KL, Lok AS, Bonkovsky HR, Di Bisceglie AM, Lee WM, Dienstag JL, Ghany MG, Gretch DR, and the HALT-C Trial Group. HCV RNA detection by TMA during the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (Halt-C) Trial. Hepatology *44: 360-367, 2006.*

National Commission on Digestive Diseases

Since its establishment by the NIH Director in 2005, the National Commission on Digestive Diseases has made substantial progress toward its goal to improve the health of the nation through advancing digestive diseases research. The Commission is responsive to the mutual interest in this research area shared by the Congress, the NIH, and the research community. Within the NIH, the NIDDK is providing leadership and support for the Commission.

As part of its charge, the Commission is assessing 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 Long-Range Research Plan that it has been tasked with developing. The Commission's efforts benefit from the diverse expertise of its members, who represent the academic and medical research and practice communities, the patient advocacy community, and the NIH and other Federal health agencies. The Commission convened for two public meetings during 2006 to initiate the research planning process through such activities as: (1) defining the 13 topic areas within digestive diseases research that make up the research plan, (2) assigning Commission members to chair 13 topical Working Groups, (3) conducting an open call for additional experts to serve as Working Group members, and (4) laying the foundation for the Working Groups' deliberations by teleconference and other means. The Working Groups are convening to identify research goals and other recommendations for inclusion in this effort. The resulting 10-year research plan will guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases. Additional information about the Commission can be found on its website: *http://NCDD.niddk.nih.gov*

Launching the New Celiac Disease Awareness Campaign

The NIDDK launched the Celiac Disease Awareness Campaign on July 18, 2006. "We now know that celiac disease is more prevalent than previously thought and that it often remains under-diagnosed," said Dr. Griffin Rodgers, Acting Director of NIDDK. "Through the campaign, we hope to increase physician awareness of the disease, resulting in earlier diagnosis and better outcomes for celiac patients."

Celiac disease is an autoimmune disorder that interferes with the absorption of nutrients from food. Individuals with this condition experience an inappropriate response by their immune systems to gluten, a protein that is found in wheat, barley, and rye, causing a wide range of symptoms such as gas, diarrhea, abdominal pain, delayed growth, skin rashes, infertility, and osteoporosis.

Once thought to be rare, celiac disease is now known to affect nearly one percent of the U.S. population. Although there is no cure, most patients can avoid symptoms of the disease by maintaining a gluten-free diet. However, because of the vast array of symptoms, celiac disease frequently goes undiagnosed.

In June 2004, the NIH held a Consensus Development Conference on Celiac Disease, sponsored by the NIDDK and the Office of Medical Applications of Research. A Consensus Statement prepared by experts from the Conference recommended that the NIDDK lead an awareness campaign for physicians, dietitians, nurses, and the public about celiac disease. Based on this recommendation, the Celiac Disease Awareness Campaign was developed, with coordination among the professional and voluntary organizations working on celiac disease. The campaign offers fact sheets, booklets, practice tools for health professionals, NIH research information, and resources from professional and voluntary organizations that focus on celiac disease.

The Celiac Disease Awareness Campaign web page, located at *www.celiac.nih.gov*, features such informational items as awareness campaign news, educational material and resources, examples of celiac disease research, celiac disease organizations, and a link to the National Digestive Diseases Information Clearinghouse.

PATIENT PROFILE

Ed McGrenaghan

Gastroparesis Compounds Diabetes Treatment

Despite having been diagnosed with type 1 diabetes at age four-and-a half and experiencing complications from the disease starting in pre-adolescence, Ed McGrenaghan, now 50, says he has been very active most of his life.

"I was involved in numerous organizations, including four Boy Scout troops and three volunteer firehouses, and I played on a number of sports teams," says Ed, who admits that getting married at age 35 slowed him down a bit. About 6 years ago, however, his health began to decline.

Part of Ed's left foot was removed as a result of an infection that would not heal due to his diabetes. About a year and a half later, on a New Year's Eve, Ed suffered a heart attack and had a stent inserted to open one of his coronary arteries. He has neuropathy damage to nerves in his feet, legs, and fingertips. Ed also has osteoarthritis and was recently diagnosed with something called adhesive capsulitis, more commonly referred to as "frozen shoulder." Although not directly related to his diabetes, the frozen shoulder is painful and difficult to treat, and the prescribed steroids can increase blood glucose levels—something that people with diabetes struggle to control.

To complicate matters even more, Ed has a condition called gastroparesis. Gastroparesis is a disabling stomach disorder that often occurs in people with type 1 or type 2 diabetes. Gastroparesis can worsen one's diabetes by making it difficult to control blood glucose levels. But thanks to research advances, Ed is finding it easier to manage both his gastroparesis and his type 1 diabetes.

Ed McGrenaghan

WHAT IS GASTROPARESIS?

Gastroparesis is a disorder characterized by symptoms of and evidence for gastric (stomach) retention in the absence of mechanical obstruction. One of the most common causes of gastroparesis is diabetes. Although not completely understood, it is thought that gastroparesis happens when the vagus nerve to the stomach is damaged and no longer controls the movement of food through the digestive system. Diabetes can damage the vagus nerve if blood glucose levels remain high over a long period of time. It is caused when high blood glucose levels injure the blood vessels that carry oxygen and nutrients to the vagus nerve. When the vagus nerve is damaged due to lack of nutrients and oxygen, the muscles of the stomach and intestines do not work properly, and the movement of food is slowed or stopped. Food that lingers too long can harden into solid masses, called bezoars, which may cause nausea, vomiting, and obstruction in the stomach. In addition, damage to the vagus nerve may account for some of the severe symptoms of gastroparesis

such as nausea, vomiting, and bloating, independent of the slowed movement of food.

When food that has been delayed in the stomach finally enters the small intestines and is absorbed, blood glucose levels rise. Since gastroparesis makes stomach emptying unpredictable, a person's blood glucose levels can be erratic and difficult to control. This problem is especially dangerous for people, like Ed, who have either type 1 or type 2 diabetes.

Signs and Symptoms of Gastroparesis

- Heartburn
- Nausea
- Vomiting of undigested food
- An early feeling of fullness when eating
- Unintentional weight loss
- Abdominal bloating
- Erratic blood glucose levels
- Lack of appetite
- Gastroesophageal reflux

LIVING WITH GASTROPARESIS AND DIABETES

Although Ed was officially diagnosed only 5 years ago, on reflection he thinks that the origins of his gastroparesis may date back almost 40 years, when he began experiencing chronic stomach ulcers at age 11. These have persisted most of his life. "At around 26 years old, I experienced frequent light vomiting for 3 or 4 months, but back then, they didn't have the diagnostic tests they have today," says Ed.

When he was in his mid-30s, Ed recalls episodes in which he would be engaged in conversation at the dinner table and had to excuse himself to rush to the bathroom, where he would have "dry heaves" and diarrhea. According to Ed, this went on for 3 years. His doctors told him that what he was experiencing was related to diabetes-induced neuropathy.

It was shortly after having had part of his foot removed 5 years ago that Ed's gastroparesis was diagnosed. "I would start vomiting at 2 a.m. and continue through to 2 p.m. the following day—and I had diarrhea at the same time," says Ed. These episodes also entailed numerous visits to the emergency room because of the dehydration that ensued. All of this was going on at the same time Ed was trying to control his blood glucose levels to keep his diabetes in check.

Ed talks about a lot of this in the past tense now, because 2 years ago, a battery-operated gastric neurostimulator was surgically implanted to help control his nausea and vomiting associated with gastroparesis. The gastric neurostimulator is an option for people whose nausea and vomiting do not improve with medications. "It's been a godsend," Ed says of the device. "It has given me back a lot of my freedom and doesn't restrict me in any way."

Since having the gastric neurostimulator implanted, Ed says that he has had only two vomiting episodes, one of which he truly believes was more viral than gastroparesis-related. However, the device doesn't do anything to relieve his episodes of diarrhea, which Ed says he still experiences about three times a month, "but they are much less severe than they used to be."

TREATING GASTROPARESIS

Unfortunately, there is no cure for gastroparesis. The primary goal for individuals like Ed, who also have diabetes, is to regain control of their blood glucose

PATIENT PROFILE

levels. Treatments include insulin, oral medications, changes in what and when to eat, and, in severe cases, feeding tubes and intravenous feeding.

Ed is acutely aware of the need to control his blood glucose levels. He has three brothers with diabetes, one of whom died of a massive heart attack associated with the disease.

Although research has produced several drugs to treat gastroparesis, Ed relies primarily on his gastric neurostimulator and an insulin pump, which he started using about 18 months before having the stimulator implanted. Prior to using these two devices, Ed's hemoglobin A1c (HbA1c) value (a measure of glucose levels over time) was in the range of 11 or 12 percent. HbA1c values provide an excellent indirect measure of how well a person's blood glucose has been controlled over the preceding two to three months, with lower values indicating better control. Since using both the gastric neurostimulator and insulin pump, the HbA1c value has dramatically dropped to 8 or 8.2 percent, he says. It is currently recommended that all persons with diabetes try to maintain HbA1c values as close to normal as safely possible (7 percent or less).

In addition, Ed watches his diet and tries to eat five or six smaller meals a day in lieu of three full ones. "I just stopped eating as much," says Ed. "As time goes on, I'm able to read myself better. As soon as I put something in my stomach I can feel it start to swell. It tells me when to stop." Ed's wife also has been a big help to him. "She works in the medical field and has access to lots of medical information. She knows what she's doing when she helps me. I couldn't ask for a better mate."

That's not to say Ed's life is back to what it was 20 years ago when he was working and volunteering his time to numerous organizations. Five years ago, Ed had to give up his job as horticulturist for a private garden center because of his deteriorating health. "Now, on a good day, I could probably work a couple of hours," says Ed, who spends most of his days at home doing household chores or taking care of others in the family, who may be ill at the time. "With all my body has been through, it just doesn't have the stamina it used to have."

On the positive side, both the gastric neurostimulator and insulin pump "bring a lot of relief," says Ed.

There is much more that needs to be learned about gastroparesis and improving its management. To address these issues, the NIDDK recently funded the "Gastroparesis Clinical Research Consortium," which consists of a network of clinical centers and one data-coordinating center working cooperatively to conduct rigorous clinical research to elucidate the functional changes associated with this disorder and to develop better treatments.

STORY OF DISCOVERY

Drug-Induced Liver Injury

Many Americans rely on approved drug therapies to manage their health problems. But in some rare cases, the cure itself can cause disease, as with adverse reactions to drugs. The liver, due to its central location and key role in processing and detoxifying foreign chemicals—including drugs—is one of the most common sites for adverse drug reactions, which can range from mild injury to acute liver failure, leading to the need for a liver transplant, or even resulting in death. Research supported by the NIH has played an integral role in advancing knowledge about drug-induced liver injury. These advances include deciphering the process by which liver cells detoxify drugs; identification of disease processes and risk factors; and revealing the magnitude of drug-induced liver injury in the U.S. due to the commonly used analgesic, acetaminophen.

Drug-Induced Liver Injury in the U.S.: While some instances of drug-induced liver injury can be traced to the inherent toxicity of a drug, many cases are unpredictable—resulting from the combination of a drug's properties and an individual's unique susceptibility. The problem is compounded by several factors, including the large number of drugs available, frequent concomitant use of multiple drugs, limitations of the current post-marketing surveillance system, and lack of specific diagnostic tests for drug-induced liver injury, which can mimic every other known form of liver disease. Beyond its significant burden on individuals and threat to the public health, drug-induced liver injury represents a major drain on the U.S. economy as the most common reason for either halting development of a promising new drug or bringing regulatory action (warnings or withdrawals) against drugs already on the market.

Understanding How Drugs Harm the Liver: Since 1923, when a therapeutic drug for gout was recognized as causing liver injury in some individuals, cases of drug-induced liver injury have risen as more drugs came on the market. From the time this phenomenon was first described, researchers have focused on determining the underlying mechanisms and disease processes at work. Over the past several decades, studies sponsored by many NIH Institutes, including the NIDDK, have made significant contributions to understanding how drugs are metabolized and how they cause liver disease.

Beginning in the 1950s, NIH-supported researchers helped to decipher the three-step process by which liver cells detoxify drugs and other foreign compounds. In "Phase 1," a complex of enzymes metabolizes the drug to a reactive intermediate. NIH-sponsored researchers working from the 1950s to 1970s contributed to the identification of a key component of this enzyme complex called cytochrome P450, and found that its activity could be influenced by genetic variations and other factors. NIH-supported research also contributed to the discovery of "Phase 2," in which the drug metabolite is inactivated by addition of a chemical group before being transported by "Phase 3" proteins out of the liver and into bodily fluids for excretion.

In the 1970s and 1980s, research performed by NIH intramural and extramural investigators using cell

STORY OF DISCOVERY

culture and animal models uncovered the mechanisms of liver injury due to specific drugs. In the case of acetaminophen—an ingredient in many over-the-counter pain medications sold in the U.S.—high levels of the reactive drug metabolite were found to be directly toxic to liver cells, resulting in cell death. Since then, further progress has been made in understanding the variety of mechanisms by which drugs can cause liver injury. Clinical research supported by the NIH since the 1980s also contributed to identifying several risk factors for liver injury caused by drugs, including age, gender, genetic make-up, nutritional status, pre-existing disease, simultaneous use of other drugs, and alcohol intake. Efforts to characterize the spectrum of disease due to drug-induced liver injury and to predict its clinical course were advanced by one of the pioneers of the field—Dr. Hyman Zimmerman at the Department of Veterans Affairs—who collaborated with NIH-supported researchers and developed a rule that predicts mortality for drug-induced liver injury associated with jaundice. A diagnostic tool consisting of a series of questions called the "RUCAM" was developed in 1989, based on recommendations from an international conference of experts. The RUCAM is still used to diagnose drug-induced liver injury, but its use is problematic, in that its interpretation varies widely, even among experts. Diagnosis continues to be based largely on exclusion of other types of liver disease, although it has benefited indirectly from NIH research on better ways to diagnose liver diseases from other causes.

Current Research Advances on Drug-Induced Liver Injury: In some cases, liver injury caused by drugs leads to acute liver failure, for which the only practical available therapy is liver transplantation. Historically, limited data were available on the causes and outcomes of drug-induced acute liver failure. The NIDDK-sponsored Adult Acute Liver Failure Study Group was founded in 1997, based on investigator-initiated efforts to address this problem by expanding knowledge about natural history, causes, and outcomes. The Group has collected samples and data needed to conduct retrospective as well as forward-looking studies that more closely examine the problem of acute liver failure in the U.S., focusing largely on cases caused by drugs.

In 2002, the Group published their ground-breaking finding that liver injury due to acetaminophen use had risen dramatically in recent years to become the most frequent known cause of acute liver failure in the U.S. Building upon this important observation, the Group recently developed an assay to directly identify cases of acetaminophen-induced acute liver failure by measuring unique compounds in the serum—an advance that could facilitate diagnosis and allow more accurate estimates of prevalence. In 2005, the Group expanded its focus to study the problem in children. The Pediatric Acute Liver Failure Study Group and the adult-focused Group now include 25 U.S. sites, where clinical trials of a therapy are being conducted to improve patient survival.

The NIDDK-led Drug-Induced Liver Injury Network, established in 2003, is another major research effort aimed at studying this health problem. Based on recommendations from an October 2000 scientific conference, this Network of five clinical centers and one data coordinating center is enabling research on liver toxicity due to prescription drugs, as well as complementary and alternative medicines. Studies aim to develop better tools for directly diagnosing,

and ultimately preventing, drug-induced liver injury, as well as enhancing knowledge of disease processes. Network investigators are currently testing a new, consensus-based diagnostic tool for drug-induced liver injury and comparing it to the RUCAM. The Network is also sharing information on cases with the FDA, and is evolving into a resource for the national clinical community and the public. Additionally, the Network is planning to identify additional cases and apply genome-wide screening techniques, which can aid in assessing the role of genetic variability. Ultimately, researchers hope to identify genetic or biological markers that indicate an individual's risk of developing drug-induced liver injury and then combine this with information on potentially toxic drug signatures. Such a body of knowledge could enable more predictive and personalized medical care in which health care providers are better equipped to determine whether a drug is safe for a particular patient or subset of patients to use.

Future Research Plans: The evolution of this field of research illustrates the continuing benefits of NIH-supported research, as well as collaboration with other Federal agencies and industry. Major NIH research programs, such as the Drug-Induced Liver Injury Network and the Acute Liver Failure Study Group, as well as investigator-initiated research efforts directed toward goals in the trans-NIH *Action Plan for Liver Disease Research,* are helping to address the problem of drug-induced liver injury.

SCIENTIFIC PRESENTATION

Translational Research on Inflammation and Colorectal Cancer

Dr. Raymond N. DuBois

Dr. Raymond DuBois is the B. F. Byrd Professor of Molecular Oncology, Professor of Medicine, Cancer Biology, and Cell/Developmental Biology, and Director of the Vanderbilt-Ingram Cancer Center. He received his M.D. and Ph.D. degrees from the University of Texas Health Science System before training in internal medicine and gastroenterology at The Johns Hopkins University Hospital, including research training with Nobel Laureate Dr. Daniel Nathans at The Johns Hopkins University Medical School, as a Howard Hughes Medical Institute Research Associate. In 1991, he accepted a position at Vanderbilt University School of Medicine as an Assistant Professor of Medicine and Cell Biology. From 1998 to 2003, he served as the Director of Gastroenterology, Hepatology and Nutrition. He has received numerous awards in recognition of his work, which focuses on colorectal cancer prevention. The following are highlights based on a scientific presentation Dr. DuBois gave at a meeting of the Institute's National Advisory Council in February 2006.

Dr. DuBois discussed colorectal cancer (CRC), and his research on its connection to inflammation. CRC is the third most common type of cancer, and also the third most common cause of cancer death in the U.S. However, the disease need not be fatal. When caught early, while tumors are still localized, survival rates are excellent. Because treatment is so much more effective when it comes early in the disease than when it comes later, surveillance is critical in older Americans, as well as in younger people who are genetically predisposed to the disease.

Of course, preventing the development of cancer, when possible, is even better than diagnosing it early. CRC can be prevented by identifying pre-cancerous growths called adenomas before they progress to overt cancer, and in some cases by altering diet or lifestyle. In addition, recent advances in our understanding of the molecular events that lead to CRC are beginning to suggest preventive approaches. Chemoprevention—the use of a drug or combination of drugs that could inhibit some cancer-related events—could be a viable option. Dr. DuBois' presentation focused primarily on this approach.

INFLAMMATION AND THE MOLECULAR UNDERPINNINGS OF CRC

Studies have shown that people who take aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) may have a lower risk of CRC than those who do not. Furthermore, inflammation caused by certain infectious diseases and other medical conditions (such as obesity) predispose people to particular types of cancer. These observations suggest a connection of inflammation to cancer that the DuBois lab and others have sought to elucidate. Inflammation may:

- Inhibit "programmed cell death" and suppress immunity, two of the body's key defenses against cancer;
- Promote growth of arteries and veins that can potentially feed a growing tumor;
- Enhance the ability of cancer cells to invade other tissues (spread); and
- Promote genetic changes that can lead to cancer.

Although inflammation has many causes, the body can trigger inflammation through production of chemicals called "prostaglandins." Cyclooxygenase enzymes are an essential component of the pathway that produces prostaglandins, and these are the enzymes that aspirin and many other anti-inflammatory painkillers act to inhibit.

Work in the last three decades has firmly established that progression from normal tissue to cancer is a multi-step process, in which a series of genetic and other changes within pre-cancerous cells remove critical constraints on dangerous cell division and proliferation. In CRC in particular, there are usually mutations in a protein known as APC. The DuBois lab and others noted that also there are frequently increased amounts of a cyclooxygenase enzyme called COX-2 present in pre-cancerous colorectal cells than in the surrounding tissue, and that even more COX-2 is often present in the cells after they have progressed to overt cancer. A larger amount of COX-2 in the cells suggests that they are potentially generating high levels of inflammatory signals, i.e., prostaglandins. These observations suggest that changes in COX-2 may be an important part of the multi-step progression to CRC, and that inflammatory signaling via prostaglandins may contribute to carcinogenesis.

Indeed, not only does reducing inflammation reduce the risk of cancer, but also high levels of inflammatory signals have been shown to enhance risk. The amount of COX-2 produced in healthy tissues is regulated, so that excessive signaling does not occur. When scientists deliberately remove this regulation in experimental animals, they create a situation in which the protein is always produced at high levels in a particular organ. Under those conditions, that organ becomes very likely to develop tumors. Additionally, animals that are genetically predisposed to develop CRC, but which lack receptors for prostaglandins, experience a markedly lower rate of growth and progression of their cancer than do mice with the same genetic susceptibility, which also have an intact inflammatory signaling pathway.

CLINICAL TRIALS USING COX-2 INHIBITORS

The clear connection between COX-2 and CRC makes the protein an excellent target for chemoprevention. The DuBois group therefore participated in a clinical trial to test a specific COX-2 inhibiting drug called celecoxib for efficacy in patients with a familial predisposition for CRC. Eighty-three patients were randomly assigned to receive either placebo or one of two different doses of celecoxib. Because their genetic predisposition is so powerful, all of the patients in this trial had a large number of pre-cancerous polyps at the outset of the study. Their polyps were counted and measured before and after the 6 month trial. Patients receiving the drug had, on average, a significant drop in the number and size of polyps compared to patients receiving the placebo. Moreover, patients taking the higher dose of the drug had a markedly better reaction than those taking the lower dose.

Interestingly, some patients had little or no response to the drug, although others responded very well—in some cases having all of their polyps disappear during the trial. A second study was later performed using blood samples taken from patients in the trial previously described, to try to detect differences between the two groups, in order to target the therapy only to those who are likely to benefit from it. In the later

study—not performed by the DuBois group—the researchers were able to determine that a particular protein was present in the blood of patients who did not respond to the celecoxib treatment, but not in the responsive patients. This type of information could be used in the future to determine who would or would not be likely to benefit from anti-inflammatory treatment to prevent or delay CRC.

Of course, people with a known familial predisposition for CRC actually represent a small fraction of total CRC cases. That is the reason it is so important for all people over the age of 50 to be screened by a colonoscopy procedure, which permits physicians to visualize and biopsy the colon for signs of cancer. Doctors can immediately remove any pre-cancerous adenomas they find during colonoscopy, which will often be all the treatment a patient needs. However, 30 to 40 percent of patients will experience re-growth of the adenomas within 3 years after such removal. Can anti-inflammatory chemoprevention improve the odds of staying cancer-free? The answer is clearly "yes," because aspirin is known to reduce 3-year recurrence rates by about 20 percent. Aspirin inhibits both COX-2 and COX-1, a related enzyme not thought to be involved in cancer.

Trials have also been under way to test celecoxib and other selective COX-2 inhibitors. Preliminary indications are that specific inhibition of COX-2 may be more effective than aspirin in preventing recurrence of adenomas. However, data made public since these trials began indicate that there is a significant risk of cardiovascular side effects in patients taking COX-2 inhibitors. Because aspirin may actually have cardiovascular benefits, it may eventually prove to be the better chemopreventive agent for some or all patients at risk of CRC. Further analysis will be necessary.

OTHER POTENTIAL THERAPEUTIC TARGETS IN THE INFLAMMATORY PATHWAY

While studies are focusing on COX-2 because it is responsible for an early step in the body's chemical process of generating prostaglandins, other investigations are exploring additional avenues to combat CRC. For example, inhibitors also exist for enzymes that promote later steps of prostaglandin production, and some of these are currently in preliminary clinical trials to test their efficacy as possible preemptors of CRC. Some of these enzymes are tissue-specific, and may potentially offer a target that is specific to the intestinal tract, thus avoiding some of the side effects associated with COX-2 inhibition.

In principle, it is also possible to interrupt inflammation at the point where the prostaglandin signal is received—at its so-called receptor. Because the COX-2 pathway generates a variety of related prostaglandins, each with a particular receptor and signaling cascade, it may be possible to finely tune chemoprevention of CRC by targeting specific prostaglandin receptors. Thus, this approach may allow interference with cancer progression, while avoiding cardiovascular problems or other unwanted side effects of COX-2 inhibitors.

Accordingly, the DuBois lab has sought to better understand inflammatory signaling downstream of COX-2. In particular, they have focused on identifying the harmful effects of a particular prostaglandin known as PGE_2. They found that administering PGE_2 over a period of time to mice with a genetic predisposition to CRC led to a dramatic increase in the number of adenomas in the animals' intestines. Most adenomas found in untreated mice in this strain of mice are in the colon, and indeed, treatment with PGE_2 increased the number and the size of the adenomas found

there. PGE_2 treatment also led to formation of a large number of adenomas in the animals' small intestines. These studies suggest that there may be potential therapeutic benefits to finding ways to inhibit PGE_2.

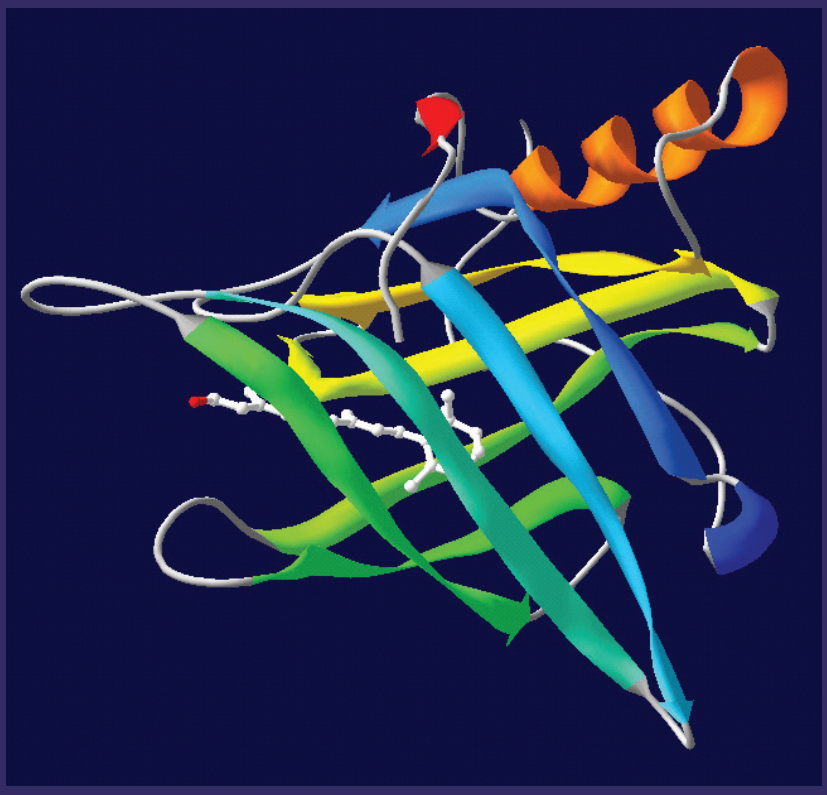
To pursue this, the DuBois group focused on a protein called PPAR-delta because its expression is typically elevated in CRC, and it is also elevated as a result of inflammation. They found that when they administered PGE_2 to mice with the genetic predisposition for CRC, but which also lacked PPAR-delta, the PGE_2 had no cancer-promoting effect. These data suggest that PPAR-delta may be a downstream effector of PGE_2, and may logically represent a potential therapeutic target.

Interestingly, PPAR-delta is also known to have a role in metabolism. Data from other groups suggest that stimulation of PPAR-delta activity can cause weight loss and improve lipid profiles and insulin sensitivity in overweight rodents. Thus, PPAR-delta stimulation may be a therapeutic option for obesity, diabetes, and hypertension, even as PPAR-delta inhibition might potentially have benefits for prevention of CRC. The DuBois lab treated the CRC-susceptible mice which had PPAR-delta with a compound to stimulate its activity. They found a significant increase in the size and number of adenomas after stimulating PPAR-delta. Whether an effect such as this would be observed in humans or even in animals that are not genetically susceptible to CRC is unknown; however, these data suggest caution is warranted when considering the advisability of either inhibiting PPAR-delta to prevent CRC or stimulating it as a treatment for other conditions. Several investigators are currently attempting to develop means of stimulating PPAR-delta to achieve benefits with respect to metabolism without side effects in the colon.

Another potential therapeutic target in the inflammatory pathway is EGFR, a growth-factor receptor that can also be stimulated by PGE_2. The DuBois lab found that inhibiting EGFR with a drug called erlotinib reduced adenoma growth and number in CRC-susceptible mice. Interestingly, they found that erlotinib combined with celecoxib, the COX-2 inhibitor, resulted in a nearly complete disappearance of adenomas in these animals. These results suggest that anti-inflammatory therapy could be combined with erlotinib or a related compound to create a chemopreventive treatment that may be more effective than either single drug in reducing the danger of CRC in some CRC-susceptible people.

CONCLUSIONS

Dr. DuBois' presentation underlined the need for effective means of preventing CRC in patients who may otherwise develop the disease. Dr. DuBois discussed the role and component parts of the inflammatory pathway within the body, and particularly within intestinal tissues. He demonstrated that inhibition of inflammation at various stages in this pathway is a viable option for preventing CRC, and finished with data showing that a combination of chemopreventive agents may be more effective than a single drug. At the same time, Dr. DuBois pointed out that the cardiovascular side effects of selective COX-2 inhibition and the positive and negative impacts of targeting particular components of the COX-2 pathway, such as PPAR-delta, are important reminders that great care must be taken in bringing new treatments into practice.



Scientists supported by the NIDDK are elucidating how specific molecules in the body contribute to obesity and its comorbid conditions, such as type 2 diabetes. One molecule of interest is a protein called retinol binding protein 4 (RBP4), produced by fat cells. A retinol binding protein is shown here as a ribbon diagram of the structure, in complex with retinol (vitamin A) shown in white and red in the center. Knowledge of a protein's structure can provide key insights into its function, and in some cases may suggest avenues for medical intervention. Other investigations into the role of RBP4 in obesity and insulin resistance are chronicled in this chapter, as well as in the chapter on "Diabetes, Endocrinology, and Metabolic Diseases."

Image created using DeepView/Swiss-PdbViewer 3.7, using data from Cowan SW, Newcomer ME, and Jones TA. Crystallographic refinement of human serum retinol binding protein at 2Å resolution, Proteins 8: 44-61, 1990.

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 non-alcoholic steatohepatitis.

Approximately 32 percent of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] 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.[3] Levels of childhood overweight have also escalated in the past several decades; approximately 17 percent of children and teens ages 2 through 19 are now overweight.[2,4] 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; 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 Acting Director of the NIDDK and the Director of the National Heart, Lung, and Blood Institute, the Task Force also includes representatives from numerous other NIH Institutes, Centers, and Offices. With extensive input from external scientists and the public, the Task Force developed the *Strategic Plan for NIH Obesity Research*, published in August 2004 (*http://obesityresearch.nih.gov/About/strategic-plan.htm*). The NIH is currently supporting a spectrum of research studies consistent with the recommendations of the *Strategic Plan.*

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

[2] *Ogden et al. 2006. JAMA 295: 1549-1555.*

[3] *Flegal et al. 2002. JAMA 288: 1723-1727; Flegal and Troiano. 2000. Int. J. Obes Relat Metab Disord 24: 807-818; Freedman et al. 2002. JAMA 288: 1758-1761.*

[4] *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.*

PRIMARY PREVENTION OF OBESITY AND KEEPING THE WEIGHT OFF

New Strategy for Fighting Obesity: A seemingly straightforward approach to avoiding excess weight gain is to eat less and exercise more, but this is difficult to accomplish. Recent research in an animal model has suggested a novel approach to helping overweight or obese people achieve that goal, through a strategy used for centuries to prevent infectious disease: vaccination. The researchers vaccinated adult rats against ghrelin, a protein hormone that signals hunger and reduces activity level. Interestingly, the ghrelin-vaccinated rats did not eat less than controls, but they did gain much less weight, suggesting that the vaccine had the effect of accelerating metabolism. It is important to note, however, that even if the effect of ghrelin vaccination is the same in people as in rats, there may be serious long-term side effects associated with vaccinating against a protein produced by the normal human body. Whether or not vaccination against ghrelin proves viable as an obesity treatment, this research is providing valuable insights into the modes of action of this intriguing hormone. It may also suggest that inhibiting ghrelin action (perhaps by developing a drug, as a potential alternative to a vaccine) could help prevent or treat obesity.

Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, Meijler MM, and Janda KD: Vaccination against weight gain. Proc Natl Acad Sci USA 103: 13226-13231, 2006.

Self-Regulation Program for Maintenance of Weight Loss: Anyone who has struggled to lose weight knows how disheartening it is to see the weight come back. A recent study has found that a face-to-face intervention incorporating daily self-weighing can help people maintain a desired weight once they have reached it. In a clinical trial, the Study to Prevent Regain (STOP Regain), investigators recruited people who had previously managed to lose at least 10 percent of their body weight, an amount that would have important health benefits. Participants were then assigned to one of three groups to assess ways of maintaining this weight loss over 18 months. One group received a face-to-face intervention; another received a similar intervention delivered through the Internet; and the third group, the control, received a quarterly newsletter. Both interventions emphasized self-regulation of body weight and daily self-weighing. Participants in each of these groups were also given a scale, and were asked to report their weight on a weekly basis. Those who had maintained weight loss ("green zone") were given positive reinforcement. Those who had regained weight ("yellow zone" or "red zone" depending upon amount regained) were encouraged to lose that weight by adjusting their eating and physical activity through the use of problem-solving skills, a tool kit provided in the study, or weight-loss counseling. This approach helped participants to catch gains in weight early and gave them a plan to help lose the weight, before the weight regain escalated. As compared to the control group, both the Internet and the face-to-face interventions reduced the risk of regaining five pounds or more, but the face-to-face intervention was particularly beneficial in decreasing the amount of weight regained. Furthermore, those who weighed themselves daily also had better weight maintenance. This study thus provides encouraging results for weight loss maintenance.

Wing RR, Tate DF, Gorin AA, Raynor HA, and Fava JL: A self-regulation program for maintenance of weight loss. N Engl J Med 355: 1563-1571, 2006.

MOLECULAR CAUSES OF OBESITY

New Insights into Molecular Signals that Influence Hunger: New research indicates that signaling by a protein known as mTOR may regulate food intake. Hunger is the evolutionarily developed message the body uses to influence feeding behavior when it needs fuel. Hunger is controlled by an array of hormones and metabolites through complex mechanisms that are gradually coming into focus through research. The mTOR protein is proving to have a central role in some of the pathways that sense nutrients and respond to metabolic hormones. Researchers found that in rats, in a part of the brain called the hypothalamus, this protein is highly active when the animals are well fed, but much less active

when they are fasting. They also found that the protein's activity is increased by experimental conditions designed to simulate certain changes in nutrient and hormone levels that can result from feeding. Further, they showed that specific inhibition of this protein's activity in this part of the brain induced rats to eat, while its stimulation reduced feeding and led to weight loss, even in rats that had been fasting. This improved understanding of the molecular control of feeding behavior could help scientists design improved approaches to treating or preventing obesity and its many associated health problems.

Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, and Seeley RJ: Hypothalamic mTOR signaling regulates food intake. Science 312: 927-930, 2006.

Molecular Mediators of Eating Behavior: Scientists are elucidating the signaling molecules in the brain that regulate eating behavior. The hope is that a better understanding of the elements that control food intake may lead to new ways to combat obesity, a complex condition arising from a combination of genetic predisposition and environmental and behavioral factors. Recent studies have uncovered some of the key molecules involved in transmitting signals from the appetite-regulating neurotransmitter serotonin. The investigators used an array of mouse models and a number of chemical agents to identify the specific subtype of serotonin receptor that mediates the decrease in food intake observed following exposure to this neurotransmitter. In further studies, the researchers found that this decrease in food intake was not seen in animals in which signaling was disrupted from another molecule—the melanocortin 4 receptor. These experiments identified this molecule as a key downstream mediator of serotonin-regulated feeding behavior. As shown by these experiments, the serotonin-melanocortin 4 receptor signaling pathway is an important component in maintaining energy balance. As serotonin analogs have been widely developed as weight-loss agents, this research may identify targets for novel future approaches to modifying food intake and controlling weight.

Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones Z, Liu HY, Zigman JM, Balthasar N, Kishi T, Lee CE, Aschkenasi CJ, Zhang CY, Yu J, Boss O, Mountjoy KG, Clifton PG, Lowell BB, Friedman JM, Horvath T, Butler AA, Elmquist JK, and Cowley MA: Serotonin reciprocally regulates melanocortin neurons to modulate food intake. Neuron 51: 239-249, 2006.

SCIENTIFIC PRESENTATION

Insulin Resistance in Obesity and Type 2 Diabetes: From Clinical Observation, to Laboratory Mice and DNA, and Back to the Patient's Bedside

Dr. Barbara Kahn

Dr. Barbara Kahn is the George R. Minot Professor of Medicine at Harvard Medical School and Chief of the Endocrinology, Diabetes, and Metabolism Division at the Beth Israel Deaconess Medical Center in Boston. She received her M.D. from Stanford University, and completed an internship, residency, and clinical fellowship in general internal medicine at the University of California, Davis. Additionally, she completed an endocrinology fellowship at the NIDDK in Bethesda, Maryland. Dr. Kahn has received numerous honors and awards, including election to the Institute of Medicine of the National Academies. Among the major research areas pursued by her research group are the molecular underpinnings of obesity, type 2 diabetes, and insulin resistance, a condition that precedes and characterizes type 2 diabetes. Dr. Kahn was invited to present her research at the NIDDK's National Advisory Council meeting in May 2006. This summary is intended to capture highlights of Dr. Kahn's talk, focusing on her exciting research on obesity, diabetes, and the RBP4 protein.

CLUES FROM CLINICAL OBSERVATIONS: THE FAR-REACHING EFFECTS OF FAT TISSUE

Dr. Kahn presented a compelling research story of "translating" biomedical research between the laboratory "bench" and the patient's "bedside." The discoveries began with an intriguing clinical observation in patients with type 2 diabetes that sparked research with animal models and DNA technology, and finally culminated in new insights from studies of patients, with implications for novel approaches to patient care.

The prevalence of type 2 diabetes in both adults and children has risen dramatically in the U.S., as has the prevalence of obesity, a strong risk factor for type 2 diabetes. The consequences of diabetes are severe, including increased risk for heart attack and stroke and reduced life expectancy, among other adverse effects on health. One avenue of scientific research to address these public health problems is the illumination, at the molecular level, of the biological defects that lead to type 2 diabetes.

Normally, Dr. Kahn explained, the body is in a state of "metabolic harmony." The pancreas, liver, muscle, fat tissue, and brain work together to make sure that fuel, in the form of glucose (sugar) or fats, is available when and where it is needed in the body, and kept at healthy levels. The body obtains glucose from food and can also synthesize it in the liver. The hormone insulin, produced by the pancreas, directs fat and muscle cells to take up glucose from the blood and can also signal the liver to dampen glucose production when necessary. In type 2 diabetes, this metabolic harmony is destroyed. The uptake of glucose from blood into the tissues is impaired because the tissues do not respond properly to insulin. This condition is called "insulin resistance." The liver also becomes resistant to insulin signaling in type 2 diabetes. Blood glucose levels thus rise too

high. In people who do not yet have type 2 diabetes, a state of insulin resistance is an ominous sign of high risk for diabetes onset.

Clinical investigators had previously observed that, in type 2 diabetes patients, most of the impairment in insulin-mediated glucose uptake is a result of defects in glucose uptake by muscle. To understand this defect, Dr. Kahn and others examined people and animals with obesity and type 2 diabetes. They assessed levels of a protein, called GLUT4, which transports glucose into cells. Surprisingly, the levels of this glucose transporter in muscle were not changed as a result of obesity or diabetes—but its levels were reduced in fat cells. This finding sparked additional research.

LABORATORY DISCOVERY: OBESITY, TYPE 2 DIABETES, AND THE RBP4 PROTEIN

To further explore how a defect in fat tissue could affect muscle and liver function, Dr. Kahn and the members of her research group developed a mouse model. They genetically engineered mice so they would lack the GLUT4 glucose transporter in their fat tissue, but not in other tissues. As would be expected, without this glucose transporter, the mice had reduced transport of glucose into fat and their fat tissue was insulin resistant. However, the muscle and liver of these mice also became insulin resistant, and the mice were at increased risk for type 2 diabetes. Something, then, must be sent out by fat cells to contribute to insulin resistance in tissues elsewhere in the body.

To discover the nature of this suspected fat-cell-produced factor, the investigators delved deeper into the molecular pathways underlying type 2 diabetes. They employed microarrays, little plates containing thousands of pieces of DNA representing the different genes in a cell. This technology enabled the scientists to examine the regulation of myriad genes of fat cells from mice that were insulin resistant as a result of the glucose transporter alteration. They homed in on a gene that was turned on at an increased level in fat cells from the insulin resistant mice. This gene encodes a protein called RBP4. The scientists then observed that RBP4 levels were also elevated in mice that were obese as a result of certain other genetic mutations, as well as in mice that became obese after eating a high-fat diet. As part of experiments to explore whether high RBP4 levels might be involved in causing insulin resistance, they injected RBP4 protein into normal mice. This subsequently caused insulin resistance.

If high RBP4 levels reflect insulin resistance, then a potential therapeutic approach for obesity or type 2 diabetes would be to try to lower RBP4 levels or block its metabolic activity. To begin exploration of this strategy, Dr. Kahn's research group turned to another aspect of RBP4: its previously-known function as a transporter for vitamin A. RBP4's name, in fact, stands for Retinol (vitamin A) Binding Protein, based on earlier research. It remains unclear how RBP4's retinol binding function might relate to diabetes. However, the knowledge of what RBP4 binds enabled Dr. Kahn and her group to obtain and test an agent that would disrupt RBP4 activity. In searching the scientific literature, they learned about a synthetic, retinol-like molecule called fenretinide that can bind to RBP4 and disrupt its function. The scientists fed mice a high-fat diet and also administered fenretinide to some of the mice. The mice that got only the high-fat chow developed elevated RBP4 levels and insulin resistance, but the mice that additionally received fen-

SCIENTIFIC PRESENTATION

retinide did not show a rise in RBP4 levels, and their insulin resistance improved. The beneficial effect on insulin resistance occurred even though the mice still gained excess weight. From these studies in mice, Dr. Kahn thought that disrupting RBP4 function could be a new strategy for preventing or treating type 2 diabetes. First, however, it was important to go back to the clinic, to investigate whether elevated RBP4 levels were associated with insulin resistance in humans.

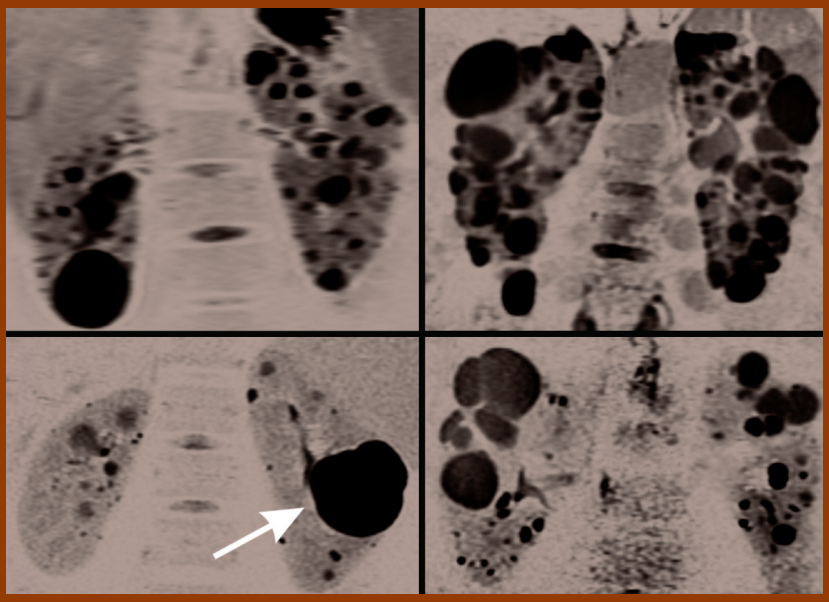
BACK TO THE CLINIC: ADVANCING RESEARCH FROM THE LABORATORY TO PATIENTS ("BENCH TO BEDSIDE")

Dr. Kahn noted that several years ago, others had reported elevated serum RBP4 levels in people with type 2 diabetes, but the meaning of these observations was unclear. With new-found insights from mice, Dr. Kahn's research group was now poised to explore in depth the association between RBP4 levels and insulin resistance in people. In collaboration with others, her research group found that RBP4 levels, measured in blood samples, were elevated in people who were obese (based on a measure of weight relative to height), and in those who were obese and had type 2 diabetes as well. High RBP4 levels also correlated with the degree of insulin resistance in these individuals. The more obese or insulin resistant their bodies were, the higher their levels of RBP4.

Next, the researchers examined the effects of exercise training in people who had been newly-diagnosed with type 2 diabetes or abnormal glucose levels. Although the exercise affected these individuals differently, those whose body's responsiveness to insulin improved the most from the exercise also had the greatest change in their RBP4 levels. Dr. Kahn's research group then sought to determine whether RBP4 might be useful as a marker of risk for diabetes. To do this, they measured RBP4 levels and insulin resistance in people who were lean and who did not have diabetes, but who were nonetheless at risk for the disease based on family history (a close relative with type 2 diabetes). In these individuals, RBP4 levels reflected the degree of insulin resistance. Additionally, the scientists found that elevated RBP4 levels occurred together with cardiovascular risk factors that are often associated with insulin resistance: high triglyceride levels, high blood pressure, a low level of the "good" form of cholesterol, and abdominal obesity. Finally, reaching back to their earlier studies of the glucose transporter protein GLUT4 in mice, the researchers assessed whether elevated RBP4 levels correlated with changes in GLUT4 levels and insulin resistance in people, and found that this was the case.

IMPLICATIONS FOR FUTURE CARE OF TYPE 2 DIABETES PATIENTS AND THOSE AT RISK

Dr. Kahn's studies have important implications for future medical care. Her research—from the clinic, to the laboratory, and back—not only suggests that a blood test for RBP4 levels may be a convenient way to assess risk for type 2 diabetes and cardiovascular risk factors, but also illuminates a potential new strategy for combating this disease: the development of drugs that could lower RBP4 levels.



Polycystic kidney disease (PKD) manifests as fluid-filled cysts in the kidneys. These cysts can progress to the point of compromising kidney function, resulting in kidney failure. Through the Consortium for Radiologic Imaging Studies of PKD (CRISP), the NIDDK is supporting research into imaging techniques capable of measuring cyst and kidney volumes to improve monitoring of disease progression. These colorized magnetic resonance images show kidney cysts (black; see white arrow pointing to one of many cysts) in four PKD patients with different ages and genetic backgrounds who participated in a CRISP study described later in this chapter.

Image courtesy of Dr. Peter Harris. Adapted by permission from Lippincott Williams & Wilkins: Harris et al. Cyst number but not the rate of cystic growth is associated with the mutated gene in autosomal dominant polycystic kidney disease, Journal of the American Society of Nephrology 17: 3013-3019, 2006.

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 sift out about two quarts of waste products and extra water from the blood, 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. As of 2004, there were 472,000 persons receiving dialysis or living with a kidney transplant.[1] Approximately 10 to 20 million people in the U.S. have earlier kidney disease.[2] 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 may lead to substantial disability and impaired quality of life. The NIDDK's urology research portfolio includes basic, clinical, and epidemiological research on the genitourinary (GU) tract. The NIDDK has supported studies in benign and noncancerous urologic disorders and diseases, including benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, pelvic floor disorders, and congenital anomalies of the genitourinary tract, as well as erectile and sexual dysfunction.

Benign prostatic hyperplasia, or BPH, is a common, symptomatic condition that increases with age in men. Prostatitis—chronic inflammation of the prostate gland—is a painful condition that accounts

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[1] *U.S. Renal Data System, USRDS 2006 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2006.*

[2] *Coresh J, et al., 2003. Am. J. Kidney Diseases 41: 1-12.*

for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genital and urinary systems. In addition to a portfolio of research studies including both basic and clinical sciences, the NIDDK sponsors the Chronic Prostatitis Collaborative Research Network, a clinical network of research sites performing clinical studies on pelvic pain of prostatitis. 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). The NIDDK's portfolio includes both basic science, as well as clinical projects. Painful bladder syndrome/interstitial cystitis (PBS/IC) is a debilitating, chronic, painful bladder disease. The number of individuals suffering with PBS/IC is not known with certainty, but it has been estimated that 700,000 to 1 million adult women in the U.S. may have the disorder, with a gender predominance of women affected (90 percent). The NIDDK sponsors the Interstitial Cystitis Clinical Trials Group/Research Network to conduct clinical studies in PBS/IC.

A conservative estimate is that approximately 13 million Americans, most of them women, suffer from urinary incontinence. Many who have the disorder still suffer in silence due to embarrassment and lack of knowledge over options available. The clinical field of urinary incontinence has changed dramatically in the last decade with the advent of new surgical procedures that have rapidly been introduced into the field. The NIDDK's Urinary Incontinence Treatment Network has produced results. Two major clinical studies have just recently been completed and are currently being analyzed.

Urolithiasis and urinary tract stone disease are frequent causes of visits to health care providers. The NIDDK has a robust interest in this field, ranging from preventative health to basic stone formation/dissolution and treatment with improvement of the current minimally invasive treatment modalities of laser or ultrasound lithotripsy or extracorporal shock wave lithotripsy (ESWL).

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. The NIDDK is conducting a clinical trial to determine if the current practice of long-term antibiotics is necessary for the treatment of these children.

The NIDDK's hematology research program uses a broad approach to enhance understanding 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.

ADVANCES IN CURBING CHRONIC KIDNEY DISEASE

Trends Show Success in Controlling End-Stage Renal Disease in People with Diabetes: End-stage renal disease (ESRD) is a costly and disabling condition that requires dialysis or transplantation for survival. It disproportionately affects racial and ethnic minority populations and is associated with a high mortality rate. Diabetes and high blood pressure remain the leading causes of

ESRD, accounting for 44 percent and 28 percent of all new cases, respectively. Data released by the NIDDK-supported U.S. Renal Data System (USRDS) indicate that rates of new cases of kidney failure have stabilized after 20 years of 5 to 10 percent annual increases. The reasons for improvement may be attributable, at least in part, to better prevention-oriented care. For patients with diabetes, the landmark Diabetes Control and Complications Trial established the importance of good control of blood glucose and the value of monitoring the urine for protein—an early sign of kidney disease. Other studies performed in the 1990s demonstrated that medications (angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) could significantly delay or prevent kidney failure, particularly in patients with protein in the urine. Unfortunately, this good news does not yet apply across the entire U.S. population. Racial disparities still persist in the rates of ESRD. The NIDDK's National Kidney Disease Education Program (NKDEP) is tailoring science-based messages to groups disproportionately affected by kidney diseases in continuing efforts to address these disparities.

Burrows NR, Wang J, Geiss LS, Venkat Narayan KM, and Engelgau MM. Incidence of end-stage renal disease among persons with diabetes—United States, 1990-2002. MMWR Weekly 54: 1097-1100, 2005.

Impact of Overweight and Obesity on Kidney Failure and Urinary Incontinence:

Researchers have reported new findings about the effects of excess weight on kidney disease and bladder control. Results from one study that analyzed data from thousands of adults have shown that the more excess weight people carried, the greater their risk for irreversible kidney failure (ESRD)—a serious condition requiring a kidney transplant or life-long dialysis. The risk associated with excess weight existed even among those who did not have diabetes or high blood pressure at the beginning of the study; both of these conditions are also known risk factors for kidney disease. One possible explanation for these findings is that those who carried more excess weight may have subsequently developed diabetes or high blood pressure, which in turn led to kidney failure. Alternatively, excess weight may also lead to kidney failure through other mechanisms. In another study, researchers found that losing a modest amount of weight, through lifestyle changes, reduces urinary incontinence in women who are overweight or obese and have pre-diabetes, a condition in which blood glucose levels are higher than normal but not yet at the point of full-blown diabetes. For this study, researchers analyzed data from the Diabetes Prevention Program (DPP) clinical trial. A major finding of the DPP, reported previously, was that people at high risk for type 2 diabetes can substantially reduce their risk for disease onset through modest weight loss, achieved through a lifestyle intervention to reduce dietary fat and calories coupled with moderately increased physical activity, such as walking. After further analysis, scientists reported this past year that the DPP's lifestyle intervention also reduced episodes of incontinence. These studies underscore the importance of obesity prevention and treatment efforts for kidney and urologic health.

Hsu CY, McCulloch CE, Iribarren C, Darbinian J, and Go AS: Body mass index and risk for end-stage renal disease. Ann Intern Med 144: 21-28, 2006.

Brown JS, Wing R, Barrett-Connor E, Nyberg LM, Kusek JW, Orchard TJ, Ma Y, Vittinghoff E, and Kanaya AM: Lifestyle intervention is associated with lower prevalence of urinary incontinence: the Diabetes Prevention Program. Diabetes Care 29: 385-390, 2006.

ADVANCES IN POLYCYSTIC KIDNEY DISEASE

Polycystic kidney disease (PKD) is an inherited disease characterized by fluid-filled cysts in the kidneys. These cysts, ranging in size from a pinhead to a grapefruit, over time can destroy functioning kidney tissue. This destructive process may result in irreversible kidney failure. There are two forms of inherited

PKD. Autosomal dominant PKD (ADPKD) is one of the most common genetic disorders and seems to affect people regardless of sex or ethnic origin. Autosomal recessive PKD is relatively rare and often causes significant mortality in the first month of life.

The NIDDK supported a clinical study, the Consortium for Radiologic Imaging Studies of PKD (CRISP), to test whether magnetic resonance imaging (MRI) of kidney and cyst volumes is a reliable early marker for monitoring disease progression. CRISP investigators used innovative imaging techniques and analyses to follow disease progression in 241 PKD patients over three years. Several important insights from this study are highlighted below.

Assessing Renal Function in Polycystic Kidney Disease: A new method using magnetic resonance imaging accurately tracks structural changes and likely enables an earlier prediction of functional changes than is possible with standard blood and urine tests in people with ADPKD. The CRISP study found that the cysts grew at a continuous, steady rate specific to each patient and that this enlargement was associated with a decline in kidney function. The CRISP study results suggest that changes in cyst and overall kidney size over time may be a reliable method of monitoring disease progression. With these new insights, researchers may be able to study agents that act earlier in the disease process, before massive enlargement of the kidneys has occurred, and thus find ways to prevent the progression of PKD patients to end-stage renal disease.

The CRISP study also found that high blood pressure, increased kidney volume, and increased cyst volume were all associated with a decline in kidney function in patients with ADPKD. In comparing various methods of estimating changes in kidney function over time, the investigators directly measured glomerular filtration rate—a measure of kidney function—using an exogenous molecule as a marker. This approach produced measurements that had the strongest association with predictors of kidney function decline.

Grantham JJ, Torres VE, Chapman AB, Guay-Woodford LM, Bae KT, King BF, Jr., Wetzel LH, Baumgarten DA, Kenney PJ, Harris PC, Klahr S, Bennett WM, Hirschman GN, Meyers CM, Zhang X, Zhu F, and Miller JP: Volume progression in polycystic kidney disease. N Engl J Med 354: 2122-2130, 2006.

Rule AD, Torres VE, Chapman AB, Grantham JJ, Guay-Woodford LM, Bae KT, Klahr S, Bennett WM, Meyers CM, Thompson PA, and Miller JP: Comparison of methods for determining renal function decline in early autosomal dominant polycystic kidney disease: the Consortium of Radiologic Imaging Studies of Polycystic Kidney Disease cohort. J Am Soc Nephrol 17: 854-62, 2006.

Linking Genetics to Disease Progression in Autosomal Dominant Polycystic Kidney Disease: Previous research showed that two mutated genes, either *PKD1* or *PKD2*, are responsible for most cases of the major form of ADPKD. However, the respective roles of these genes in disease progression, as indicated by ultrasound analysis, have remained unclear. The CRISP investigators, using a more sensitive MRI method, reported that patients with the *PKD1* gene have more cysts and significantly larger kidneys than those with the *PKD2* gene. Data from the CRISP study suggest that this difference results from earlier development of cysts, not from a faster growth of cysts, in patients with *PKD1* mutations. These clinically important results will inform the development of targeted therapies for patients with the most prevalent form of this disease.

Harris PC, Bae KT, Rossetti S, Torres VE, Grantham JJ, Chapman AB, Guay-Woodford LM, King BF, Wetzel LH, Baumgarten DA, Kenney PJ, Consugar M, Klahr S, Bennett WM, Meyers CM, Zhang QJ, Thompson PA, Zhu F, and Miller JP: Cyst number but not the rate of cystic growth is associated with the mutated gene in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 17: 3013-3019, 2006.

UNDERSTANDING THE DEVELOPMENT OF RED BLOOD CELLS

An iron transporter has been found to play an important role in the development of red blood cells and could have significant implications in the treatment of various anemias. Working with zebrafish as a model organism, researchers found that mutant fish with severe anemia were unable to produce red blood cells and had mutations in a specific gene (*mitoferrin,* or *mfrn*). This gene encodes a protein that imports iron into a specialized compartment within developing red blood cells where the molecule heme is synthesized. Heme is the iron-containing component of the molecule hemoglobin, which delivers oxygen in the blood to the tissues of the body and carries away carbon dioxide. The mutations prevented the protein from transporting iron into this compartment and impaired the synthesis of heme—thereby inhibiting the maturation of zebrafish red blood cells. This resulted in severe anemia, which was usually fatal.

Red blood cells derived from mouse embryonic stem cells missing this same gene, and hence the iron transporter, showed a similar failure to develop into mature red blood cells. Furthermore, the gene and its encoded protein appear to be functionally conserved across species; for example, introduction of a normal mouse *mfrn* "rescued" the mutant zebrafish, restoring their ability to produce red blood cells. A corresponding gene has been found in humans, and it is possible that mutations in it may play a role in some congenital forms of anemia in people. The discovery of this gene and its cognate protein provides an important tool for researchers studying iron metabolism and red blood cell development, as well as a potential future therapeutic target.

Shaw GC, Cope JJ, Li L, Corson K, Hersey C, Ackermann GE, Gwynn B, Lambert AJ, Wingert RA, Traver D, Trede NS, Barut BA, Zhou Y, Minet E, Donovan A, Brownlie A, Balzan R, Weiss MJ, Peters LL, Kaplan J, Zon LI, and Paw BH: Mitoferrin is essential for erythroid iron assimilation. Nature *440: 96-100, 2006.*

National Kidney Disease Education Program (NKDEP)

An estimated 10 to 20 million Americans currently suffer from chronic kidney disease (CKD) and, according to the NIDDK-supported United States Renal Data System (USRDS), more than 330,000 patients are on dialysis. Patients with CKD are at increased risk for kidney failure. It is estimated that treating the number of people with kidney failure, also called end-stage renal disease (ESRD), through dialysis or kidney transplantation now costs the U.S. health care system more than $30 billion every year. ESRD is an enormous public health problem that disproportionately affects minority populations.

To help address these issues, the NIDDK supports the NKDEP. This educational program seeks 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 progression from CKD to kidney failure can be prevented or delayed if it is detected and treated early enough. The NKDEP underscores that effective treatments and management strategies for kidney disease exist, yet are being underutilized.

The Program uses several strategies to help achieve its goals. Toward this end, it is implementing public education and awareness initiatives; creating tools and programs for health care providers who play a role in diagnosing and treating CKD 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.

Examples of current and recent activities of the NKDEP include:

- *Outreach to People with Diabetes and High Blood Pressure:* In addition to working with specific racial and ethnic populations, the NKDEP is a direct resource to those at risk for CKD due to the two main risk factors—diabetes and high blood pressure. The NKDEP has developed a new, easy-to-read brochure entitled, "Make the Kidney Connection." This brochure encourages people with diabetes and high blood pressure to talk to their health care providers and get tested. Because many people with these conditions are not aware of their risk for kidney disease, and therefore would not think materials on kidney disease relate to them, this brochure focuses on diabetes and high blood pressure to attract their attention. The brochure, suitable for a diverse population, was reviewed by an expert panel and audience-tested through focus groups and in a real-world setting.

- *Outreach to Family Members of Dialysis Patients:* Several years ago, the NKDEP developed materials entitled, "Help Your Family Prevent Kidney Failure," to encourage dialysis patients to talk to family members about their risk of developing kidney disease. To optimize distribution of these materials, "The Dialysis Center Working Group" was convened to advise the NKDEP regarding how best to interact with dialysis centers. The working group meeting prompted a joint mailing with the Forum of ESRD Networks to dialysis centers to inform dialysis patients about NKDEP materials and other relevant materials from the

NIDDK's National Kidney and Urologic Diseases Information Clearinghouse.

- *Creatinine Standardization Program:* The NKDEP's Laboratory Working Group is leading an effort to reduce inter-laboratory variation in creatinine assay calibration, which is critical to improving the accuracy of estimates of glomerular filtration rate (eGFR)—a key measure of kidney function. The new recommendations on measuring serum creatinine provide an opportunity to detect CKD earlier. Launched in 2006, the program encourages *in vitro* diagnostic manufacturers to recalibrate serum creatinine methods to those that are traceable to "perfect" standards in measurement procedures and support customer laboratories in the transition from using current methods to those that are standardized. The Laboratory Working Group's recommendations for creatinine standardization were published in the January 2006 issue of *Clinical Chemistry*.

- *2006 Family Reunion Initiative Kicks Off:* The NKDEP has kicked off the second year of its African-American Family Reunion Initiative. The goal of the initiative is to encourage African-American families to discuss the connection among diabetes, high blood pressure, and kidney disease at reunions and other family gatherings. This year, the NKDEP has expanded the Kidney Connection Guide—to include additional tips for planners and fact sheets on diabetes and high blood pressure.

- *New Spanish-Language Initiative:* The NKDEP launched a new Spanish-language initiative to raise awareness about risk factors for CKD among Hispanic Americans. The initiative includes a website and brochure that highlight the connection between kidney disease and its primary risk factors—diabetes and hypertension. Hispanics are disproportionately affected by diabetes and hypertension, and are nearly twice as likely to develop kidney failure as non-Hispanic whites. The website and brochure provide science-based information on the risk factors for kidney disease, the basic principles of kidney function, as well as the importance of early testing. The materials stress that someone can have kidney disease without knowing it. The materials also stress the availability of medications that can prevent or slow the disease progression. Both resources offer additional Spanish-language resources on diabetes, hypertension, and kidney disease. The new materials were developed in collaboration with kidney disease experts and dialogue in Spanish with community-based organizations serving the Hispanic community. To view the NKDEP Spanish-language website, and to download or order the brochure, visit *www.nkdep.nih.gov/espanol*

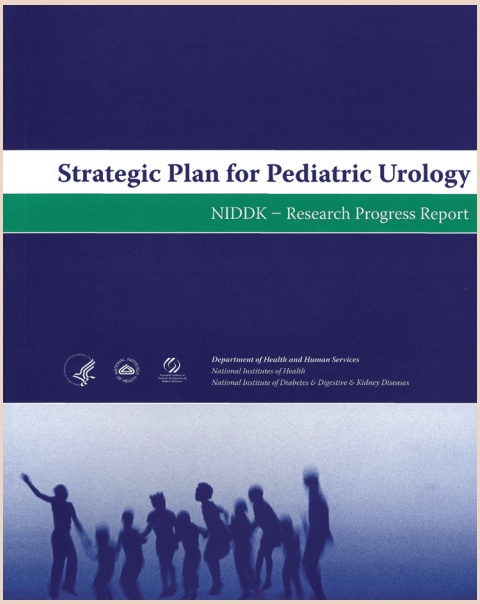
Through continued educational efforts, the NKDEP contributes to helping primary care providers better assess, treat, and educate patients about CKD; to improving the use of diagnostic tools by health care practitioners; and to coordinating Federal efforts in this area.

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

A Strategic Plan for Research in Pediatric Urology

The Strategic Plan for Pediatric Urology, NIDDK—Research Progress Report, published in February 2006, was developed under the Institute's auspices with the contributions of external experts in urology. The Plan's Executive Summary conveys the many challenges intrinsic to pediatric urological diseases, for example, the need to discriminate between children at risk for severe long-term complications requiring intervention and the larger group who are not; the complexity and variety of the congenital anomalies; and the need for multicenter clinical trials to study the relative infrequency of many conditions. In addition, the Plan addresses topics such as the health impact of pediatric urological diseases, the major clinical needs in pediatric urology; the clinical presentation and treatment of these diseases; and basic, clinical, and translational research priorities.

An example of one of the diseases highlighted in the Plan is vesicoureteral reflux (VUR), a disease of the lower urinary tract. VUR results from a developmental defect in which abnormal insertion of the ureter into the bladder causes retrograde flow of urine into the ureter and the upper urinary tract. Consequently, patients are at an increased risk for urinary tract infections leading to kidney damage or scarring. In follow-up to recommendations in the Plan, the NIDDK is about to launch the Clinical Study of Vesicoureteral Reflux in Children, a trial which aims to recruit a group of approximately 600 affected children to determine which ones are likely to benefit from long-term antibiotics and which ones are at the highest risk for scarring of the kidneys and progression to kidney failure.



The *Strategic Plan for Pediatric Urology, NIDDK—Research Progress Report*, published in February 2006, identifies priorities for research on urological conditions that affect children. The report was developed by the NIDDK with input from external experts in the field. It can be accessed on the NIDDK website at: *http://www.niddk.nih.gov/federal/planning/Pediatric-Urology/*

PATIENT PROFILE

Andrea Arnold

Coping with Chronic Kidney Disease at Age 13

It's 7:15 on a Wednesday morning. While her friends are heading off to school, 13-year-old Andrea Arnold's grandmother has just dropped her off at the outpatient dialysis unit at Children's Hospital. By the time Andrea settles in, is weighed, has her temperature and blood pressure checked and her heart and lungs assessed by the unit's staff, it's around 8:00 a.m.

As she makes herself comfortable in a medically designed reclining chair, one of the staff members inserts two needles into the vascular access on Andrea's upper right thigh. One needle carries Andrea's blood to the dialyzer, a medical device that will remove the harmful waste products that accumulate in her blood because doctors had to remove her kidneys; the other needle returns the cleansed blood to her body. Andrea winces a bit when the needles are inserted. It's one of the most unpleasant parts of hemodialysis. For the next 3 hours and 15 minutes Andrea will sit back in the recliner while the dialysis machine rhythmically whirrs and beeps, pumping and cleansing her blood and removing excess fluid from her body to keep her alive. This routine takes place 3 days a week.

Cora Dixon tutors Andrea while she's being dialyzed. "Andrea's a good student and should be an attorney," says Ms. Dixon, who adds that Andrea asks very challenging questions, especially about her health. Unfortunately, many of the answers would be daunting for an adult, let alone a 13-year-old.

Andrea Arnold

For the next 3 hours and 15 minutes Andrea will sit back in the recliner while the dialysis machine rhythmically whirrs and beeps, pumping and cleansing her blood and removing fluid from her body to keep her alive.

CHRONIC KIDNEY DISEASE

Andrea has reflux nephropathy, which occurs when a faulty one-way valve-like system allows urine to flow back from the bladder up to the kidneys. Because the pressure in the bladder is generally higher than in the kidneys, the reflux of urine exposes the kidneys to unusually high pressure. Over time, this increased pressure will damage the kidneys and cause scarring, which could lead to chronic kidney failure and

PATIENT PROFILE

end-stage renal (kidney) disease, as it did with Andrea. Andrea was diagnosed with urinary reflux to the kidney when she was 6 months old, acute kidney disease at the age of 3 years old. She progressed to chronic kidney disease at the age of 9. At age 12, she had both of her kidneys removed. Reflux nephropathy can run in families. Andrea has a 14-year-old brother, André, but fortunately, he shows no signs of the disease.

About four out of 1,000 children have reflux, but show no symptoms. Up to 50 percent of infants and children with urinary tract infections have reflux. It is estimated that reflux nephropathy may cause as many as 20 percent of the cases of renal failure in children and young adults. Young girls are especially prone to infections because their urethra, which is the tube leading from the bladder to outside the body, is relatively short compared to that of boys. This shortness makes it easier for germs from outside the body to make their way to the bladder.

Andrea also had a condition called chronic pyelonephritis. Pyelonephritis is infection and inflammation of the kidneys. The combination of reflux and bladder infections exposes the kidneys to the possibility of pyelonephritis. *Chronic* pyelonephritis is a long-standing infection that does not clear and can, over the years, increasingly damage the kidneys. It, too, can cause end-stage renal disease. In Andrea's case, it necessitated the removal of both her kidneys.

To compound matters, Andrea has an enlarged heart as a result of the hypertension from her kidney disease. Although she takes several medications, she's allergic to one of the antibiotics commonly used in the treatment of reflux. She also has asthma, further complicating her treatment.

LIVING WITH CHRONIC KIDNEY DISEASE

In many ways, Andrea's entire childhood has been defined by her disease. At just 6 weeks old she was hospitalized with a bladder infection. Three months later, young Andrea was back in the hospital with another bladder infection. By age 6 months Andrea was diagnosed with reflux and once again hospitalized. "It just continued to get progressively worse," says Andrea's mother, Patrice Arnold.

A year after being diagnosed, Andrea had her ureters, which carry urine from the kidneys to the bladder, surgically reimplanted into her bladder to help stop the reflux and to diminish the frequency and severity of her kidney infections. In all, Andrea has undergone seven surgeries related to her kidney disease. One required her to wear an appliance (urostomy bag) for many years, until her kidneys were removed. As a result of her kidney disease, Andrea's body has difficulty maintaining an appropriate balance of the minerals calcium and phosphorus, and her bones are weak.

According to Andrea, the worst thing about having this disease is that it has stunted her growth. "I grow slower than most kids my age," says the precocious adolescent. Andrea weighs just under 90 pounds and is four feet, nine inches tall. "I wish I were a bit taller," she says.

Andrea claims that she feels little, if any, pain as a result of her current condition, but her kidneys "hurt a lot" from the time that she was 6 years old until they were removed 6 years later.

Because their blood types match, Andrea's mother is hoping to be able to donate one of her kidneys to her daughter. For now, Andrea must carefully monitor her calcium and phosphorus levels and follow a strict diet, a difficult assignment when you're 13 years old and want to be like every other 13-year-old.

"I'd just like to do more things with people my age," says Andrea, "like eat French fries, chocolate, ice cream, dark sodas (colas), tomatoes, fresh fruit," all of which she is restricted from eating because of their high levels of sodium, phosphorus, or potassium. Andrea also says that she liked to dance "before my bones got too weak." If she were healthier, she'd like to play basketball. "I'd get to run and play with the other kids," she says.

"I'd just like to do more things with people my age," says Andrea, "like eat French fries, chocolate, ice cream, dark sodas (colas), tomatoes, fresh fruit," all of which she is restricted from eating…."

In the meantime, while she waits in hope that she will be able to receive a kidney from her mother, Andrea will remain on hemodialysis 3 days a week, as she has for the past 2 years.

"I do most of my homework when I'm on the machine (dialyzer)," says Andrea. When she's not being tutored, volunteers come in to entertain her and the other kids in the dialysis unit with crafts and painting. "I've made friends with the other children here with kidney disease," says Andrea. "We don't see each other as sick. We're just close friends and we talk about everything." But she quickly adds that she'd rather be home, in school, or at the mall than being dialyzed.

It's nearly 11:30 on this particular Wednesday morning. Amid the quiet bustle of the outpatient dialysis unit, Andrea will once again be weighed, have her temperature and blood pressure taken, and her heart and lungs assessed before she leaves for the day. Today, she says, she feels fine, but there are days when she's a little dizzy or weak after her dialysis. She will be taken home to her great-grandmother and grandmother's house, where Andrea will either finish whatever homework she has left or lie down and take a nap until her mother gets off from work to pick her up later this evening. It's already been a long day for this courageous 13-year-old.

When asked if she has a message for kids her age, Andrea replies without hesitation "that you should never feel sorry for yourself. There's always someone worse off than you."

PATIENT PROFILE

Hope Through Research

The NIDDK supports a range of programs and studies devoted to improving treatment for patients like Andrea and others with progressive kidney disease and permanent kidney failure, including those on hemodialysis.

- The NIDDK—along with several other Institutes at the NIH—has established the Chronic Kidney Disease in Children (CKiD) study to recruit over 500 children with mild to moderately decreased kidney function and follow them for 5 years. Researchers will study risk factors for further decrease in kidney function; closely monitor brain development; examine risk factors for heart disease; and look at the long-term effects of poor growth in this group.

- The NIDDK is about to launch the Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) clinical trial. This trial aims to recruit a group of children with vesicoureteral reflux to determine which ones are likely to benefit from long-term antibiotics and which ones are at the highest risk for scarring of the kidneys and progression to kidney failure.

- NIDDK's End-Stage Renal Disease Program promotes research to reduce medical problems from the complications of kidney failure and to improve the effectiveness of dialysis and transplantation.

- The upcoming Hemodialysis Vascular Access Clinical Trials Consortium will conduct a series of clinical trials of drug therapies to reduce the failure and complication rate of vascular access in hemodialysis.

- The U.S. Renal Data System (USRDS) collects, analyzes, and distributes information about the use of dialysis and transplantation to treat kidney failure in the United States. It also helps identify problems and opportunities for more focused special studies of kidney research issues.

STORY OF DISCOVERY

Ironing Out the Anemia of Inflammation

In a feat of scientific serendipity, researchers have now discovered that a molecule first found in a search for antimicrobials lies at the crux of the anemia of inflammation—the second most common form of anemia.

How Various Forms of Anemia Can Result from Low Iron Levels: Anemia is a potentially serious health condition that occurs when the blood suffers a drop in its capacity to deliver oxygen throughout the body. Iron is essential to the body's oxygen-delivery system. Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. Because it can be dangerous to have too much iron (as well as too little), total body iron is carefully regulated, with most of it being constantly recycled. While small amounts are absorbed daily via the digestive tract, about 10 times more iron is simply retrieved from aged red blood cells and reused. Special cells in the liver and spleen digest red blood cells and release their iron to the blood. A protein called transferrin picks up this iron, along with the dietary iron that has been absorbed via the digestive tract. Transferrin then carries the iron to the bone marrow, where it is used to make new red blood cells. If insufficient iron flows to the bone marrow, however, normal red blood cell production drops and anemia can result. Without treatment, even mild anemia stresses the body, which tries to compensate for reduced oxygen levels by increasing heart rate and respiration. Thus, one important cause of anemia is iron deficiency, the most common cause of anemia, which can be corrected through administration of iron supplements. Another form of anemia, however, is associated with inflammation.

Understanding the Anemia of Inflammation and Chronic Disease: The anemia of inflammation and chronic disease affects people who have infections, chronic inflammatory disorders—such as rheumatoid arthritis—and many chronic disorders, including cancers. It may also occur with acute, critical illnesses. While this form of anemia resolves if the underlying condition resolves and is not usually severe, it can contribute to a poorer prognosis for affected patients. Patients with this form of anemia typically have inadequate red blood cell production, low blood iron (hypoferremia), and low levels of transferrin; they are also resistant to the effects of erythropoietin, the hormone that normally stimulates and regulates red blood cell production. Patients with the anemia of inflammation and chronic disease are usually not iron-deficient. Instead, the iron balance in their bodies has been altered, such that more iron is sequestered in the cells involved in iron recycling and absorption, as well as in liver cells that store iron. This cellular sequestration of iron leaves less available for transport to the bone marrow. Attempts to treat the anemia of inflammation and chronic disease with oral iron supplements typically do not work, even though this form of anemia mimics iron deficiency. Rather than being used for red blood cell formation, the new iron is simply added to the cellular stores. Thus, a major mystery has been why these cells do not relinquish sufficient iron to restore iron flow to normal and thus avert anemia.

For several decades, scientists studied the effect of inflammation and chronic disease on iron metabolism, but these studies were not able to fully explain the iron

STORY OF DISCOVERY

sequestration. Now, a series of fortuitous discoveries by NIH-supported researchers and others has solved part of the puzzle with the discovery of hepcidin.

Role of Hepcidin: Hepcidin was identified 6 years ago in a search for small molecules active in "innate immunity," the body's first-line of defense against invading bacteria, fungi, and other microorganisms. Examining urine and blood samples from humans, researchers found a small, hairpin-shaped molecule structurally similar to antimicrobial molecules in other species. To their surprise, however, the gene encoding the molecule—later named hepcidin—was expressed predominantly in the liver. This finding took an important turn when a research team studying a condition called "iron overload" found that liver production of hepcidin increased in mice given excess iron. A rapid succession of studies in animal models and humans followed. From this research, hepcidin emerged as a fundamental regulator of iron balance that inhibits iron absorption and iron release from tissue stores when iron levels are high, and eases off when iron levels decline.

How does hepcidin contribute to anemia of inflammation and chronic disease? It turns out that hepcidin production is not only stimulated by increased iron levels, but also by inflammation. Researchers studying hepcidin found that if they induced inflammation in mice, liver production of hepcidin quickly rose. At the same time, blood iron levels fell—similar to what is seen in this form of anemia. This inflammatory response was ablated, however, if the mice were hepcidin-deficient, or if they lacked a certain inflammatory molecule, called cytokine IL-6. These findings indicated that, in response to IL-6, hepcidin mediates inflammation-induced drops in plasma iron.

Reinforcing these results in mice, a clinical study showed that IL-6 administration led to a rapid rise in hepcidin levels and drop in plasma iron in people. Concomitantly, research showed that people with anemia of inflammation have elevated levels of hepcidin in their urine—thus confirming that hepcidin has an important role in causing this health condition.

Scientists have also uncovered the way that hepcidin likely causes the drop in plasma iron. Cells handling iron transport have several ways to obtain iron, but only one way to export it, through a so-called "transporter protein" in the cell membrane called "ferroportin." In experiments with cultured cells, hepcidin binds to ferroportin and induces its destruction, thereby preventing iron from exiting cells. This key finding explains why iron accumulates in cells that store iron, but is not released for use by the bone marrow.

Thus, hepcidin-induced sequestration of iron in response to inflammation explains—in whole or in part—how affected patients can be replete with iron but, ironically, cannot use it. Scientists are still grappling with the question of why this state is permitted to continue, given that iron pathways in the body normally try to correct anemia. Indeed, in animal models, other forms of anemia normally decrease hepcidin expression, thus increasing iron flow. Yet, in the anemia of inflammation and chronic disease, the interaction between IL-6 and hepcidin overrides other body signals and causes and maintains anemia.

Therapeutic Possibilities: Consistent with hepcidin's role as a regulator of iron balance, the dysfunction or dysregulation of this molecule is implicated in a number of iron overload disorders. Recent studies suggest that pathways important in iron overload may

provide a promising route for preempting hepcidin's role in anemia of inflammation. Special attention has focused on one form of inherited iron overload, "juvenile hemochromatosis." In humans, mutations in the gene encoding a protein called hemojuvelin result in this severe, early-onset iron overload. A recent study showed that mice genetically engineered to lack hemojuvelin not only exhibited iron overload, but also had reduced hepcidin levels and increased ferroportin. These results showed that hemojuvelin is important in regulating the hepcidin pathway. Subsequently, scientists determined that there are two forms of the hemojuvelin protein—a longer, cell-associated form, and a shorter, "soluble" form found in blood. While the full-length hemojuvelin protein appears to positively regulate hepcidin gene expression, the soluble form exerts a negative effect on hepcidin levels and, most importantly, inhibits hepcidin stimulation by the cytokine IL-6. Thus, researchers are exploring the possibility that soluble hemojuvelin may provide a therapeutic approach with which to modulate abnormal hepcidin levels in the anemia of inflammation and thereby to correct the anemia.

This rapid progress in identifying hepcidin and discovering its role at the intersection of inflammation and iron metabolism has been critical to progress in understanding the anemia of inflammation and chronic disease. While it has yet to be determined whether excess hepcidin is sufficient to cause this form of anemia, its key role in the underlying biologic process has been firmly established.

While much has been learned, an intriguing research question remains: why would the body choose to exacerbate illness by causing anemia when a patient is sick? Iron is not just essential for human life; it is also used by invading microbes and rapidly growing tumor cells. Researchers speculate that this "iron sequestration response" developed during evolution because it favored survival from infections, for when the body "hides" iron and prevents its further uptake via the digestive tract, it establishes a defense mechanism that hinders the growth of infectious organisms. However, this defense mechanism incurs a cost by causing anemia. Moreover, it is misdirected in patients with chronic inflammatory conditions (such as rheumatoid arthritis), and it is inappropriately active in older persons in the absence of any infection or malignancy. Researchers will need to consider the potential harm or benefit of changing iron availability in different conditions associated with the anemia of inflammation and chronic disease as new discoveries about hepcidin are translated into new therapies for this common form of anemia.

SCIENTIFIC PRESENTATION

Tipping Iron Balance

Dr. Nancy Andrews

Dr. Nancy Andrews is a leading researcher in the field of iron biology. Her research group focuses on iron transport pathways involved in systemic iron homeostasis, and the elucidation of molecular mechanisms underlying diseases of iron uptake and deficiency. Dr. Andrews is the George R. Minot Professor of Pediatrics and the Dean for Basic Sciences and Graduate Studies at Harvard Medical School, a Senior Associate at Children's Hospital Boston and a Distinguished Physician of the Dana-Farber Cancer Institute. The following are highlights based on a scientific presentation Dr. Andrews gave at a meeting of the Institute's National Advisory Council in September 2006.

Dr. Andrews focused her presentation on genes and cellular pathways that she and her collaborators have discovered are directly involved in inherited diseases of toxic iron overload, known collectively as "hereditary hemochromatosis." She then turned her attention to her group's recent search for modifier genes that affect these primary pathways—and, hence, can also tip overall iron balance. Through a wealth of fundamental studies, she and her research team are finding and fitting together various pieces of the puzzle of iron homeostasis that will help point the way to new approaches to treating iron overload disorders.

IRON HOMEOSTASIS

Dr. Andrews began by explaining iron homeostasis, or the regulation of iron movement and metabolism that leads to stable iron levels in the body. Iron is an essential element absorbed from the diet. Absorption is normally very tightly regulated, because there is no specialized mechanism for excreting iron, which can become toxic in high amounts. Iron is only lost from the body through bleeding and through the shedding of skin cells and cells lining part of the digestive tract. Only a small amount of iron (1 to 2 milligrams) enters the body every day, to balance or only slightly exceed these small daily losses.

Some of the body's iron circulates in a form bound to the plasma protein, transferrin. This iron is primarily delivered to the bone marrow, where it is incorporated into hemoglobin in developing red blood cells. About two-thirds of the iron in a healthy individual is found in red blood cells and their precursors. Over time, old or damaged red cells are engulfed by cells called tissue macrophages, which break them down and "recycle" their iron, returning it to transferrin for reuse. Iron is also used by all other cells and tissues in the body, in very small amounts. The remaining body iron is generally deposited in the liver. Normally, total iron content in the human body is about 2 to 4 grams.

HEREDITARY HEMOCHROMATOSIS

Hereditary hemochromatosis is a disease that develops because of increased dietary iron absorption, and it is characterized by increased serum iron levels. Excess iron is deposited in a variety of tissues, where it catalyzes the formation of toxic oxygen species (free radicals) and in that way causes tissue damage. The primary target organs for deposition of excess iron include the liver, where iron loading results in fibrosis, cirrhosis, and a markedly increased predis-

position to hepatocellular carcinoma; the heart, where it is associated with cardiomyopathy and arrhythmias; and in endocrine tissues such as the pancreas, where it disrupts normal function and may impair insulin secretion and cause diabetes.

The small intestine is the gatekeeper for the absorption or exclusion of dietary iron. To enter the circulation, dietary iron must be picked up on the apical (top) side of cells lining the small intestine, and then be released through their "feet," or the basolateral side. Over-absorption of iron could thus result from excess iron being picked up on the apical side (and then dumped out the basolateral side), or from dysregulated iron release on the basolateral side. To understand iron transport pathways in the absorptive cells lining the small intestine, Dr. Andrews' research team took advantage of the knowledge that vertebrates are very similar in how they deal with iron. Thus, by studying rodents and other animals with heritable iron transport defects, they could then use powerful genetic techniques to identify the genes affected, and hence the molecules responsible.

One iron transporter discovered through these studies was ferroportin. Iron transport defects lead not only to iron overload, but also to iron deficiency, and hence, anemia. A fellow scientist studying severe anemia in a zebrafish mutant animal model strain (*"Weissherbst"*) identified a mutant gene that was implicated in basolateral iron transport. The gene encoded ferroportin. A collaborative research effort ensued and led to the extensive characterization of mouse ferroportin in the Andrews lab. Ferroportin is present in the cell membrane of intestinal cells and macrophages, and is the only known "iron exporter" in cells. Because of its likely role in human hemochromatosis, Dr. Andrews' lab pursued studies of its regulation.

While these studies were under way, the serendipitous discovery of "hepcidin" by other researchers yielded another key piece of the puzzle of how iron homeostasis is controlled. Hepcidin is a small protein that controls serum iron concentration by regulating export of iron recycled from old red cells to the serum, and also by controlling intestinal iron absorption. Hepcidin is produced almost exclusively by the liver, in varying amounts that depend on iron needs.

With the key observation that inherited iron overload is associated with low levels of hepcidin, studies of ferroportin and hepcidin became linked. Research soon revealed that hepcidin inhibits ferroportin activity by binding to it and causing its degradation. These findings led to an updated model for iron homeostasis. In this model, ferroportin activity is normally regulated by hepcidin, such that some iron stays in the intestinal cells or in the macrophages, and some is released. In hemochromatosis, however, low hepcidin levels lead to increased ferroportin activity—i.e., increased iron export—and, in turn, to the increased serum iron that causes deposition of iron in the liver, heart, and elsewhere. This model provided the springboard to the next steps in studying iron overload—determining where genes implicated in different forms of hemochromatosis fit into the iron puzzle.

COMPLEXES IN IRON REGULATION

Dr. Andrews explained that there are two types of inherited hemochromatosis. Adult onset hemochromatosis is relatively mild and can be

caused by mutations in the ferroportin gene, a gene called *HFE*, or a gene called *TFR2*. Juvenile, or early-onset, hemochromatosis is more severe and can be caused by mutations in the hepcidin gene, or in the most recently discovered hemochromatosis disease gene, *HJV*, which encodes the hemojuvelin protein.

Studies in mouse models, as well as examination of patients, soon indicated that these hemochromatosis-inducing mutations actually fall into three functional "classes": mutations in the ferroportin gene, which change the function of this iron exporter; mutations in the hepcidin gene, which directly affect its regulation of ferroportin; and mutations in the other genes (*HFE*, *TFR2*, and *HJV*), which lead to reduced hepcidin levels—indicating that these three genes are involved in regulating hepcidin. *HJV* mutations exert the most severe effects on hepcidin levels, while *HFE* and *TFR2* mutations have milder effects, which suggests that they act to "fine tune" hepcidin production. Thus, an important goal for the Andrews lab has been to work out how the proteins encoded by these three genes regulate hepcidin—most importantly, the role of hemojuvelin.

A break came in deciphering hemojuvelin's role when studies by another NIDDK-supported investigator suggested that hemojuvelin and similar proteins might act as co-receptors for bone morphogenetic proteins, or BMPs. This was exciting because BMP proteins are involved in regulating expression of a wide variety of genes, including genes affecting growth, development, and bone. BMPs interact with specific receptors on the cell surface to stimulate a signaling cascade inside the cell that ends with changes in gene expression. "Co-receptors" can help direct, refine, or amplify BMP-induced changes in gene expression in different cell types by interacting with a BMP and/or its receptor.

A series of collaborative studies in mouse models and in cultured cells indicated that hemojuvelin indeed acts as a co-receptor in a BMP pathway. Through a set of elegant experiments, Dr. Andrews and her colleagues showed that hemojuvelin forms a complex with BMP and a BMP receptor to initiate a signaling cascade that leads to hepcidin expression in liver cells. This key finding was followed by studies suggesting that the HFE protein takes the same molecular journey—that HFE joins the hemojuvelin-BMP-BMP receptor complex when iron levels are high and it is "freed" from its association with another iron regulatory protein (TFR1). Moreover, biochemical experiments showed that the third putative hepcidin regulator, TFR2 protein, interacts with HFE and also becomes part of the complex when iron levels are high. TFR2 may also play an important role in keeping the entire complex stable. Thus, at least these three proteins—and possibly more—appear to be involved in a large regulatory complex that helps modulate BMP signaling for hepcidin production.

So why make the signaling complex so complicated? Dr. Andrews proposed that this assemblage allows for a rheostat-like regulation of hepcidin expression. In her model, the absence of the hemochromatosis-associated proteins means that BMP-induced hepcidin levels are minimal. Adding just hemojuvelin back to the complex restores much of its activity and provides a very large induction of hepcidin expression. However, in order to have full production in the face of increasing iron levels, HFE and TFR2 may be required.

MODIFIER GENES THAT AFFECT IRON BALANCE—ALTERING THE IRON SET POINT

In the most common form of hemochromatosis, caused by *HFE* mutations, there is great variability in how severely the disease affects human patients. In contrast, genetically identical mice with *HFE* hemochromatosis do not show such variability. This observation suggests that there are modifier genes in human populations that affect the clinical expression of this disease. Such genes modify the so-called iron set point, and are important to understanding both iron pathways and the course of iron overload disease in individuals.

To look for modifier genes, Dr. Andrews' research team approximated the human situation by comparing the iron levels of different mouse strains—i.e., of mice that are not genetically identical. The researchers could later use powerful genetic tools to isolate the relevant differences. Upon examining iron stores in the liver and spleen (the primary site of red blood cell iron recycling) in nine different mouse strains, the team found substantial variation. In particular, two of the strains exhibited very large differences in iron content. The researchers then performed a genetic mapping experiment: "low iron" and "high iron" mice were bred together. The offspring were then bred with each other, creating a so-called "F2 generation." The purpose of this breeding was to generate mice whose chromosomes were patchworks of chromosomes from the "pure" strains—permitting researchers to more readily "map" characteristics, such as differences in body iron levels, to particular chromosomes.

The "patchwork" F2s exhibited a very wide range of liver and spleen iron content. This indicated that multiple genes were likely involved in the iron differences. Turning the data from those F2 mice into chromosomal positions, they found that liver iron characteristics were associated with four different chromosomes. In contrast, most of the difference in spleen iron between the two strains was associated with mouse chromosome 9.

Focusing on chromosome 9 and spleen iron content, Dr. Andrews explained how her research team used molecular tools and more genetic mapping methods to gain further insights. Going back to the original mouse strains, they introduced smaller and smaller pieces of chromosome 9 from the "high iron" mouse strain into the genome of the "low iron" strain. By performing these manipulations and then looking at the effect on spleen iron content in the resulting mice, they were able to narrow down the candidate segment of chromosome 9 from a region encompassing hundreds of candidate genes to a region encompassing just a few. A single candidate gene, "gene B," eventually emerged from this study. By analyzing the DNA sequence of gene B from the two mouse strains, they found that a unique genetic variation could explain why the "low iron" mice are so different from other strains.

Using molecular tools, the team studied the protein product of gene B in the "low iron" and "high iron" mice. They initially detected this protein, "protein B," inside iron-recycling macrophages from the mouse spleen. However, the amount of protein B didn't appear to differ between "high-" and "low iron" mice. Thus, the team wondered whether, instead, protein B influences proteins involved in iron accumulation. The iron exporter ferroportin was a good candidate. What they found when they compared spleen ferroportin levels between the two mouse strains was that spleen macrophages from "low iron" mice had more

ferroportin on their surface (and hence more exit points for iron) than those from "high iron" mice—consistent with the reduced accumulation of iron in the spleens of "low iron" mice. While the exact mechanism has yet to be worked out, these initial studies suggest that protein B is involved in some way in membrane trafficking of ferroportin, independent of hepcidin.

SUMMARY AND FUTURE STUDIES

Hereditary hemochromatosis is a genetic iron overload disease that can be caused by different genes and ranges in severity. Mutations in the hepcidin gene cause hemochromatosis because no functional hepcidin protein is produced. Mutations in ferroportin can, in some cases, cause hemochromatosis because the mutant ferroportin is no longer regulated appropriately by hepcidin. Mutations in the other three hemochromatosis-associated genes are thought to cause hemochromatosis by inactivating components of a BMP signaling complex involved in inducing hepcidin expression.

Within *HFE* hemochromatosis, there is variability in disease severity among individuals. The Andrews lab is searching for genes that might modify hemochromatosis severity in mice as a way to discover why the severity of hemochromatosis varies in people. Gene B and its protein product are likely candidate hemochromatosis disease modifiers.

Future studies will focus in detail on what the function of protein B is, and whether variants in gene B actually influence hemochromatosis in mouse models—and, if so, whether there are disease-relevant gene B variants in human patients. Dr. Andrews briefly described exciting studies being pursued by young investigators in her lab. These focus on candidate modifier genes for liver iron content, and candidate serum proteins that modify hepcidin expression. She closed by noting that, as a principal investigator and in her position as the Dean of Basic Sciences and Graduate Studies, her greatest concern is keeping extremely talented young investigators involved in science.

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