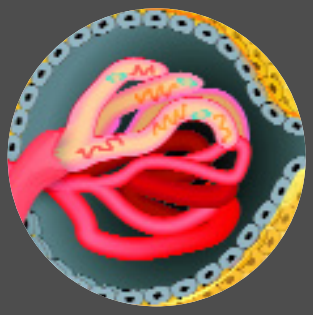
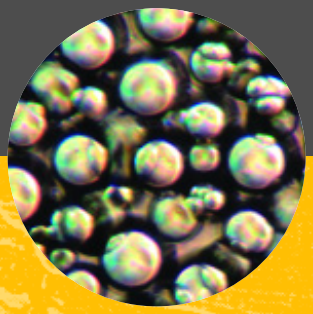
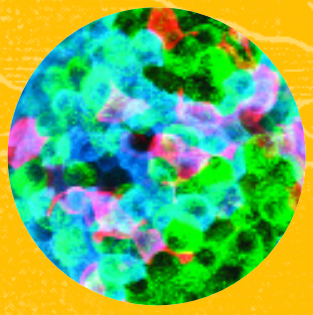
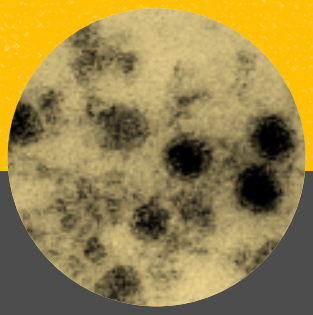
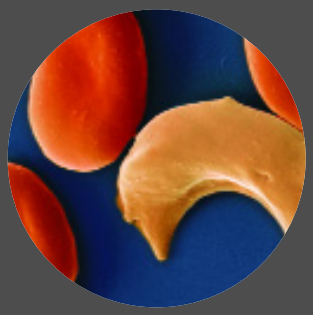










NIDDK

Recent Advances & Emerging Opportunities

February 2003

National Institute of Diabetes & Digestive & Kidney Diseases

National Institutes of Health

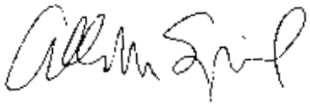
Message from the Director

The research mission of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) encompasses a wide array of chronic diseases and other serious illnesses affecting tens of millions of Americans. As we review recent successes and set new goals for effectively combating these diseases, we at the NIDDK are pleased to present this document, our third annual compendium of highlights in NIDDK-supported basic and clinical research. Over the past year, researchers supported by this Institute have continued to expand knowledge about basic cellular and molecular processes, and to capitalize on this knowledge to improve treatments for people suffering from many debilitating diseases. We are steadily uncovering and characterizing the complex molecular pathways involved in the development of type 1 and type 2 diabetes, obesity, digestive and liver diseases, kidney disease, urologic disorders, and blood diseases. In turn, we are also seeing the translation of these basic research advances into clinical applications through vigorous efforts to take research from "the bench to the bedside."

The examples of research advances collected in this booklet represent a substantial return on the significant investment that has been made in research by Congress and the President over the past several years. As we move into the future, this commitment to basic and clinical research will provide fertile ground for researchers and tremendous scientific opportunities. This is truly an exciting time for biomedical research. For example, every day we learn more about how genes interact in complex ways with each other and with the environment either to promote or to protect us from disease. Furthermore, revolutionary changes in computing and biotechnologies are providing researchers with ever more powerful tools with which to approach basic and clinical research problems. The advances contained in this booklet tell an important part of this story.

Along with research advances, we have included brief essays about five leading scientists and their visions of the future of research with respect to diabetes, obesity, digestive diseases, kidney disease, and urologic diseases. As in past years, we also feature several "Stories of Discovery," which are intended to illustrate how today's science advances are built on a strong foundation of past research accomplishments. Within these pages you will also find profiles of several people affected by diseases such as type 1 diabetes, inflammatory bowel disease, and interstitial cystitis. These stories illustrate the ways in which NIDDK-supported research is affecting the lives of patients, and they remind us that the ultimate purpose of biomedical research is to preserve health and to benefit people touched by disease.

No single publication could capture the sweeping breadth and depth of new knowledge and insights that have been gained during the past year. Rather, our intention is to provide an overview of some of the major advances, along with a discussion of why they are of particular significance. It is our hope that you will find these advances an exciting and promising reflection of the NIDDK's many contributions to the national biomedical research enterprise.



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

CONTENTS

In mouse embryos, NIDDK researchers have demonstrated that the Edg1 gene is vital to maintaining the integrity of developing blood vessels. The mouse embryo on the left has normal Edg1 gene expression. The mouse embryo on the right lacks Edg1 and has leaky blood vessels, causing hemorrhages in the placenta and the embryo. By using knockout mice to gain an understanding of basic developmental processes, scientists will shed light on developmental problems that lead to diseases and disease complications. Photo: Dr. Richard Proia, NIDDK.

Cross-Cutting Science: Paving the Way to Discovery

Advances in medicine are largely dependent upon the accumulation of new knowledge about biologic processes, especially at the smallest levels of an organism—its genes, the proteins they encode, and the workings of cells. While the ultimate application of such basic research is not always obvious, major strides in fighting disease can be traced back to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Described here are some recent studies of fundamental processes, ranging from the development of cells to the development of organisms, and the technologies that make such studies possible. The insights gained through this type of research can be expected to propel disease oriented research, not only within the NIDDK mission, but also in many other fields. Investment in such cross-cutting scientific research today will have future applications that we cannot now describe with certainty, but which we know will surely be realized.

MOUSE MODELS OF METABOLIC DISEASES AND DEVELOPMENTAL PROCESSES

In the quest for new and better treatments for disease, biomedical scientists are creating and using exciting advances in modern technology to discover important genes and learn their functions at an ever-increasing rate. This knowledge can propel new advances in diagnostics and drug development. Because different diseases and medical conditions arise from disparate causes—mutations in genes, infectious agents, and environmental factors—scientists delve into the inner workings of living cells with a great diversity of approaches. In genetic research, genes are often identified, and clues to their function obtained, by investigating what goes wrong when a gene is mutated. The study of genes has been revolutionized in recent years by modern functional genomics—the use of large-scale, high-throughput techniques to discover the function of genes and how all the genes in the genome of an organism work together.

An equally important tool for studying gene function is the use of model organisms. Scientists can create a model of a disease caused by a mutation in a known gene, or study how a gene mutation that has been characterized in a single cell affects a whole organism, by generating an animal with an analogous mutation. From the animal model, they can learn how a disease or a developmental process progresses and what other genes may be involved. They can also use these animal models to test candidate disease therapies that are not yet ready for human trials. Mice have been particularly useful for such studies because of their small size, rapid reproduction, and large numbers of offspring per litter, allowing researchers to perform informative experiments quickly. More significantly, mice and humans share virtually the same set of genes and the DNA sequence of the mouse genome is therefore an essential tool to identify and study the function of human genes. In fact, the recently-obtained draft sequences of the human and mouse genomes indicate that the two are approximately the same size and are about 85 percent identical.

Moreover, the differences involve only a few hundred of the 35,000 or so genes in both organisms. The complete sequences for both are now being assembled. Because of this high degree of similarity, it is believed that much research into mouse models of human disease has application to humans.

Mice are relatively easy to modify genetically, either through direct genetic engineering or through selective breeding. Mouse embryonic stem cells can be manipulated in culture and then implanted in female mice to produce animals that lack a specific gene entirely—a traditional gene "knockout"—or that lack the gene only in certain tissues or only under certain conditions—a so-called "conditional knockout." (See section, "Further Developments in Stem Cell Biology," for more information about stem cells.) Both approaches contribute valuable information about the normal function of a gene by allowing scientists to observe the consequences of its absence.

A complementary approach to gene knockouts is the generation of "transgenic" mice through injection of foreign DNA into developing mouse embryos. This technique produces mice that possess a gene that they normally would not possess, or that turn on a gene at times or in tissues where it normally would be silent. Researchers can then study the impact of a gene where it is normally not present as a way to gain insight into the gene's function. Genes suitable for this kind of study are not limited to mouse genes, as genes isolated from a wide range of organisms are functional in mice.

Once derived, such knockout and transgenic animals may be simply interbred to produce mice with multiple genetic alterations. To date, literally hundreds of knockout and transgenic mouse lines have been derived, many possessing multiple genetic alterations. Such mouse models have provided important insights into the development and treatment of many diseases. As illustrated by the following examples, researchers continue to make interesting and sometimes unexpected discoveries about gene functions and their role in both normal and disease states using mouse models.

Cell Signaling Pathways and Blood Vessel Formation: As an embryo develops, blood vessels are generated, a heart begins to beat, and blood begins to flow. Each cell generated during development appears to have a set of instructions that tells it where to go and what to do. Cell signaling plays an essential role in facilitating the developmental process. Such signals are often transmitted between cells through small molecules, called ligands, that are released by one cell and "seen" by another cell. The second cell "sees" the signal because it has on its surface a molecule specifically designed to bind to the signaling molecule: a molecular "receptor." Binding of the ligand to its receptor launches a cascade of molecular events within the target cell.

NIDDK researchers, working in collaboration with other researchers, have studied the role played by ligand and receptor-mediated signaling in the development of the circulatory system, specifically the blood vessels. As blood vessels form during embryonic development, the central "tube" of a vessel forms first. Muscle cells then migrate to surround the immature vessels, providing the strength and support they require. These researchers created a knockout mouse that does not possess the cell surface receptor known as endothelial differentiation gene-1, or Edg-1. The ligand for this receptor is a fat-derived molecule, sphingosine-1-phosphate.

When the researchers surveyed mouse litters in order to characterize the effect of knocking out Edg-1, they were unable to find any mice with two copies of the inactivated gene (each gene in each cell is present in two copies, or alleles: one from the father and one from the mother). Further examination revealed that the knockout mice were not viable: at about day 13 of development, Edg-1 knockout mice hemorrhage and die. The researchers determined that hemorrhaging is due to malformed blood vessels. Without Edg-1, the muscle cells do not completely envelop the vessels as they should, leaving them weak and vulnerable to hemorrhaging. These studies revealed that the Edg-1 receptor is required for blood vessel formation during embryonic development and that the signaling pathway is essential for mammalian development. Previous research suggests that this pathway may play important roles in adult blood vessel development and stability as well. If this is so, Edg/sphingosine-1-phosphate pathways may be involved in blood vessel development during wound healing and solid tumor growth. Thus, they present potential therapeutic targets for treating injury and disease, such as the blood vessel damage sustained as a complication of diabetes (see chapter on "Diabetes, Endocrinology, and Metabolic Diseases").

Cell Signaling and Insulin Resistance: All cells have a membrane that serves as a barrier between the cell and its surroundings. The cell membrane is composed of a fluid lipid (fat) bi-layer containing molecules and structures that facilitate interactions and communication between the cell and its environment. Lipid and protein molecules "swim" along the surface of this bilayer, forming raft-like clusters of molecules and receptors that constitute specialized "microdomains." The microdomains, in turn, can help direct cellular activity by regulating the transmission of signals to the cell's interior so that the cell can respond properly.

Gangliosides are molecules composed of lipid and sugars that are present on the surface of mammalian cell membranes. These molecules play a variety of roles in cell signaling, both as modulators of receptor cell signaling and as ligands—molecules that bind receptors. One molecule in particular, the GM3 ganglioside, can influence the responsiveness of the insulin receptor to its ligand, the hormone insulin. NIDDK researchers and their collaborators have studied the role of GM3 in insulin signaling by generating mutant mice in which the gene that synthesizes this ganglioside is knocked out. Mice that lack the gene necessary to synthesize ganglioside GM3—and, consequently, that lack GM3—are generally healthy. However, the mutant mice are more responsive to insulin than normal mice and respond to insulin by reducing their blood glucose levels more rapidly. In the mice lacking GM3, insulin receptor activity in skeletal muscle is elevated, suggesting that one function of GM3 gangliosides is to regulate insulin receptor activity.

When placed on a high fat diet, normal mice become overweight and develop glucose intolerance as evidenced by their decreased ability to respond to insulin. The researchers found that GM3 knockout mice were better at lowering their blood glucose levels and were more sensitive to insulin, even though they gained as much weight on the high fat diet as the normal mice did. Thus, GM3 may play a role in the insulin resistance resulting from high-fat diets that is seen in normal mice. Based on these findings, it is possible that inhibiting expression of the GM3 molecule could be a potential treatment for insulin resistance in type 2 diabetes.

Tay-Sachs and Sandhoff Diseases: Tay-Sachs disease and a related disorder, Sandhoff disease, belong to a large group of inherited disorders known as lysosomal storage diseases. Storage disorders are caused by a defect in one of the enzymes in the lysosome, the intracellular "recycling center" in which old or damaged molecules are broken down. If one of these disposal enzymes is missing or non-functional, intermediate breakdown products accumulate, and the disposal pathway becomes "backed up" at one particular point. The accumulation of partially degraded molecules within the lysosome ultimately causes damage to the cell. Tay-Sachs and Sandhoff diseases are members of a subcategory of lysosomal storage disorders known as GM2 gangliosidoses. These disorders are so named because they result from an inability to break down completely GM2 ganglioside, a molecule found in nerve cell membranes. Children with Tay-Sachs disease lack the enzyme beta-hexosaminidase A (Hex A), while children with Sandhoff disease lack Hex A and the related enzyme Hex B. In the absence of Hex A, partially degraded gangliosides accumulate in brain cells, resulting in brain damage and ultimately causing death.

Research using animal models has suggested that an intense inflammatory response may contribute significantly to the neurodegeneration seen in Tay-Sachs disease. The "Sandhoff mouse" lacks Hex A and serves as a model for both Tay-Sachs and Sandhoff diseases. Using a technique called DNA microarray analysis, researchers have previously found that a large number of genes that are expressed at elevated levels in the mouse model are related to an inflammatory response.

Researchers working at the NIDDK and their collaborators have now extended these findings to humans. Using tissue samples from a patient with Tay-Sachs disease, a patient with Sandhoff disease, and an unaffected child as a control, they generated a "profile" of gene expression patterns from all three patients using a technique known as serial analysis of gene expression (SAGE). The SAGE profiles show significant increases in expression of genes regulating pro-inflammatory responses, consistent with what was observed in the Sandhoff mouse. These results were confirmed by examining microscope slides of the tissue sections. Significantly, many of the genes identified in these studies have been proposed to play roles in Alzheimer's, Parkinson's, and other neurodegenerative diseases. This finding suggests that a common mechanism of brain cell damage may play a major role in these diverse diseases.

Mouse models continue to be an important approach to exploring gene function in health and disease. To facilitate development, validation, and sharing of information about mouse models of diseases within its mission, the NIDDK has initiated several collaborative research efforts. These include the "Mouse Models of Diabetic Complications" consortium, which seeks to refine or derive accurate mouse models of human diabetes complications for use by the research community for a variety of investigations, including the testing of therapeutic, prevention, early detection, or imaging strategies; and "Mouse Metabolic Phenotyping Centers for Models of Diabetes and Its Complications," to allow detailed characterization in mouse models of sometimes subtle metabolic changes associated with disease complications. Another current initiative will support individual projects for the development of mouse models to identify genetic modifiers of disease loci.

Myerowitz R, Lawson D, Mizukami H, Mi Y, Tifft CJ and Proia RL: Molecular pathophysiology in Tay-Sachs and Sandhoff diseases as revealed by gene expression profiling. *Hum Molec Genetics* 11: 1343-1350, 2002.

Liu Y, Wada R, Yamashita T, Mi Y, Deng C-X, Hobson JP, Rosenfeldt HM, Nava VE, Chae S-S, Lee M-J, Liu CH, Hla T, Spiegel S and Proia RL: Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J Clin Invest* 106: 951-61, 2000.

Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, Kono M, Tsuji S, Daniotti JL, Werth N, Sandhoff R, Sandhoff K and Proia RL: Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc Natl Acad Sci USA* (in press, 2003)

CONTENTS

ORPHAN NUCLEAR RECEPTORS IN HEALTH AND DISEASE

One critical aspect of cell signaling is the modification of gene expression. Molecules inside and outside the cell can change how genes are expressed—and hence the activities of a cell—by influencing the activities of a class of hormone receptors known as nuclear receptors (NRs).

Nuclear receptors are active in the nucleus at the center of the cell, as opposed to the cell surface membrane. They play an important role in endocrinology, contributing to regulation of development, metabolism, and disease. Nuclear receptors function in response to hormones, dietary lipids, xenobiotics (drugs), and other known and unknown metabolites. Most function primarily as transcription factors, proteins that affect how well a gene is expressed by modulating the transcription of a gene's DNA sequence into the messenger RNA that is eventually translated into a protein. The biology of NRs is complex, with cellular localization, tissue specificity, and gene target action dependent on a myriad of factors, including so-called "accessory proteins" in the nucleus and the DNA of the target gene(s). Nuclear receptors may bind to DNA singly (monomers) or in organized aggregates (dimers and heterodimers), with or without ligand. The presence or absence of ligands can define interactions with key regulatory proteins that determine whether a gene will be repressed or activated. Thus, over the long-term, the activities of NRs can have profound effects on cellular activities involved in obesity, development, metabolism, diabetes, heart disease, osteoporosis, and hormone-dependent cancers.

In particular, the Orphan Nuclear Receptor subfamily (ONR) of NRs has moved to the forefront in studies on lipid and drug metabolism. Although classified as orphans, i.e., without known hormone ligands (in contrast to the traditional nuclear receptors such as the estrogen, androgen, and thyroid hormone receptors), the ONRs respond to a variety of dietary lipids, xenobiotics, and natural metabolites. While considerable information exists on the structure and function of many of the nuclear receptors, far less is known about the ONRs. Not only can increased knowledge of the ONRs inform our understanding of basic metabolism, but it will also serve as the basis for the development of therapeutics to treat metabolic diseases, such as obesity and osteoporosis. For example, a major class of insulin-sensitizing drugs works through an ONR, PPAR-gamma. Greater knowledge of this cell signaling mechanism has the potential to lead to improved therapeutic agents for diabetes.

Through a series of grants and cooperative agreements, the NIDDK has fostered efforts designed to lead to a greater understanding of receptor specificity, ligand selectivity, interaction with cytoplasmic and/or nuclear accessory proteins, chromatin, and the transcriptional machinery, as well as identification of the downstream target genes regulated by the ONRs. Individual investigator-initiated efforts funded by the NIDDK have already led to numerous research advances that are elucidating the roles of ONRs.

Central to efforts to unravel the roles of ONRs in health and disease is the "Functional Atlas of Orphan Nuclear Receptors," a trans-NIH cooperative agreement spearheaded by the NIDDK. The NIDDK, five academic institutions, the National Institute on Aging, and the National Cancer Institute have set a goal to develop this unique database to acquire, catalogue and integrate information on ONRs. The long-term goal is to delineate the role(s)

of ONRs in normal and pathophysiological conditions, including obesity, osteoporosis, hormone dependent cancers, processes of aging, and diabetes and its complications. The objective of the Atlas is to provide useful data to researchers in the scientific community in a timely fashion for use in their own investigations relevant to ONRs. The hope is that the Atlas will serve as a catalyst for progress in this area by leveraging ongoing efforts to elucidate the structure and function of ONRs. It is anticipated that the Atlas will enhance coordination with emerging information from the human (and other) genome effort(s) and catalyze progress toward development of greater understanding of ONR function and dysfunction, ultimately leading to better therapies for treatment of disorders of metabolism.

FURTHER DEVELOPMENTS IN STEM CELL BIOLOGY

As body cells wear out and die, stores of adult stem cells help to regenerate these functionally-compromised cells and the tissues they constitute. With the hope of one day treating diseases effectively through cell-based approaches, scientists are exploring the capability of undifferentiated cells, called "stem cells," to be coaxed into specialized cells of the body, including pancreatic, brain, liver and other types of cells. This line of research has also been called "reparative medicine."

Although promising, this field requires extensive fundamental studies to understand how stem cells function and react to their environment—an exciting pursuit which will enlighten scientists about the steps stem cells take as they differentiate, committing themselves to different developmental fates whereby they become the myriad tissues in the body.

There are several different types of stem cells under study. "Adult" stem cells are rare populations of undifferentiated cells found in the tissues of adult animals and humans. Studies have shown that adult bone marrow, which feeds the body's circulatory system, may be a good source of these cells. Another type of stem cells, called "embryonic" stem cells, may also be derived from either animal or human tissue. Consistent with the policy announced by President George W. Bush on August 9, 2001, the NIH only funds research involving human embryonic stem cells if it is in accordance with criteria established by the Administration.

Because research on all these types of cells is still in its earliest stages, scientists cannot predict in advance which of them may prove most effective and appropriate for therapeutic purposes. Thus, they are working diligently to characterize these cells and understand their workings. Clearly, many questions remain to be answered. For example, it is not fully known how stem cells accomplish their dual tasks of self-renewal (to generate more stem cells) and of differentiation into specialized cells—the two traits that define their "stemness."

From Stem Cells to Specialized Cells: In one recent study, NIDDK-supported scientists analyzed genes that are turned on (expressed) in mouse embryonic stem cells and adult neural and blood stem cells, but not in differentiated cells. Of all of the genes expressed in the stem cells, a core set of 216 genes was shared in common among all three stem cell varieties; this set of genes likely gives stem cells their "stemness." Scientists can now further investigate these genes.

As stem cells go through development to produce specialized cells, they first generate progenitor cells. Progenitor cells are intermediates in stem cell differentiation that retain varying degrees of "potential" to become various specialized cell types. The degree of potential depends upon their developmental stage. A team of NIDDK-supported scientists recently identified adult bone marrow progenitor cells from humans and rodents that appear to have differentiation potential rivaling that of embryonic stem cells. The scientists first identified these cells, called multipotent adult progenitor cells (MAPCs), in human bone marrow, demonstrating that these cells could be coaxed to differentiate into a variety of specialized cell types, including bone cells, fat cells, blood vessel cell types, and nervous system cells. Adapting the techniques they developed for human cells, the scientists then extricated MAPCs from the bone marrow of mice and rats to facilitate later experimentation in animal models.

To explore further the differentiation potential of MAPCs, the research team investigated whether these cells could morph into yet another cell type—liver cells. They isolated MAPCs from the bone marrow of human donors and from mice and rats, and tried growing the MAPCs in different ways to coax them to differentiate into liver cells. They then screened the differentiated cells for liver-specific biological markers and subjected the cells to a battery of functional assays; the tests revealed that the MAPCs could differentiate to acquire a distinct set of liver cell traits, adding another cell type to their repertoire.

Next, the scientists demonstrated that these different specialized cell types did in fact arise from a single "multipotent" cell, and were not simply descendants of several different less-potent progenitor cells—crucial evidence for the existence of MAPCs. By inserting special genetic tags into mouse MAPCs to uniquely mark the DNA of each MAPC and all cells derived from it, the scientists were able to trace the lineage of diverse specialized cell types back to the same original MAPC. Similar experiments with human MAPCs confirmed that they, too, are multipotent.

The scientists also showed that both human and rodent MAPCs can grow and divide extensively in the laboratory, repeatedly doubling their numbers while maintaining their differentiation potential. Further, despite these generations of cell divisions, the MAPCs did not show molecular signs of "aging." Finally, the scientists investigated the differentiation potential of MAPCs in an animal. When put into mice, mouse MAPCs developed the characteristics of specialized cells from a variety of tissues and organs, but did not form tumors in animals—an occurrence that has been associated with some embryonic stem cells.

In theory, clinicians could eventually develop therapies that use MAPCs retrieved from a patient as back-up cells, ready to adapt and replace damaged tissue anywhere in the body. The use of a patient's own cells would also eliminate risks associated with transplants from donors. By bringing to light the remarkable differentiation potential of MAPCs, these studies open new opportunities for stem and progenitor cell research and research on cell-based therapies.

Understanding the Physiology of Hematopoietic Stem Cells: Blood cells are regenerated by adult hematopoietic stem cells (HSCs), which reside primarily in the bone marrow. The generation of blood cells—both red and white—is termed hematopoiesis. Several recent studies have suggested that some HSCs can also differentiate into non-hematopoietic cell types—findings that are still under investigation. Importantly, the crucial role of HSCs in regenerating the blood supply in bone marrow transplant patients and their potential for use in gene therapy in genetic diseases of the blood (see "Kidney, Urologic, and Hematologic Diseases" chapter) have propelled research to understand the physiology of HSCs.

In bone marrow transplantation, suspensions of cells extracted from donor marrow are injected into the bloodstream of a patient whose own defective or diseased marrow cells have been ablated through radiation or chemotherapy. To be successful, a small subset of the donor cells, the most "primitive" HSCs—called multipotent long-term HSCs—must repopulate or "engraft" into the recipient's bone marrow and establish a new, renewable source of blood cells. The question is, how do these cells get from the bloodstream to the marrow—by random chance or through physiologically relevant mechanisms for cell migration?

To answer this question, researchers examined whether they could find evidence for HSC migration in normal, healthy mice. They physically joined the circulatory systems of pairs of healthy mice whose blood cells could be distinguished by a subtle difference in one cellular marker. The mice were paired this way for various lengths of time. After separating the pairs, the researchers assayed for the functional cross-engraftment of long-term HSCs as evidenced by their sustained contribution to each mouse's blood supply. They found that the cells successfully cross-engrafted in at least one partner from each pair, generating up to 3.7 percent of peripheral blood cells in mice joined for 7 weeks. Longer periods of joining increased the percentage of engrafted cells directly detectable in bone marrow. These findings indicate that long-term HSCs from the circulation can engraft into normal, healthy bone marrow, and not just replace absent bone marrow cells. Furthermore, the research team found that the engrafting activity of an enriched fraction of HSCs and progenitor cells can be reduced if the cells are first "filtered" through the circulatory system of another mouse—suggesting that a mechanism(s) does indeed exist for rapid removal of HSCs from the circulation.

In related work, researchers from the same laboratory defined another marker for HSCs, one that distinguishes between long-term HSCs and short-term HSCs (which maintain self-renewal for only 8-to-10 weeks). Through cell-sorting and bone marrow reconstitution assays, they determined that, in addition to previously known markers, the absence of a receptor tyrosine kinase on the cell surface (Flk-2) distinguishes long-term from short-term HSCs. They also found that the concomitant loss and gain of two cell surface markers coincides with the loss of self-renewal in HSC maturation.

These findings suggest that there are normal physiological mechanisms for HSC migration between the circulation and the bone marrow. They also provide a tool to enable researchers to better isolate particular populations of HSCs, including long-term HSCs. As indicated, bone marrow transplantation is a serious procedure for both donor and patient. These research results may have long-term implications for improved therapy, facilitating less-risky isolation of long-term HSCs from the circulation rather than from bone marrow for use in transplantation. They also have important short-term implications for the enrichment and study of long-term HSCs and other HSCs.

Christensen JL and Weissman IL: Flk-2 is a marker in hematopoietic stem cell differentiation: A simple method to isolate long-term stem cells. *Proc Natl Acad Sci USA* 98: 14541-6, 2001.

Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, and Verfaillie CM: Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418: 41-9, 2002.

Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, and Melton DA: "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* 298: 597-600, 2002.

Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu W-S, and Verfaillie CM: Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 109: 1291-302, 2002.

Wright DE, Wagers AJ, Gulati AP, Johnson FL and Weissman IL: Physiological migration of hematopoietic stem and progenitor cells. *Science* 294: 1933-6, 2001.

ONGOING AND NEWLY LAUNCHED NIDDK EFFORTS

The NIDDK supports cross-cutting, basic research endeavors through funding for investigator-initiated projects and ideas and for multiple Institute-led initiatives aimed at accelerating research in particular areas. The NIDDK is also responding to the enormous opportunities provided by advances in information technology, as well as biotechnology, to establish scientific resources that can be accessed by the research community at large. This response includes plans to establish an "NIDDK Data and Bisosample Repository"—a central facility for archival storage of biosamples and data collected in large, multi-site studies, and for processing of genetic samples. This repository will enhance the value of large studies by increasing access to the biosamples and data that have been collected and by facilitating efficient sharing of these resources. This resource will be enormously useful for both basic and clinical research scientists.

Furthermore, large-scale genome anatomy projects (GAPs) for multiple tissues and organs—including the pancreas, gut, and hematopoietic cells—have been or are being created by the NIDDK to develop and apply genomic approaches to basic and disease-related research areas. The GAPs will support the use of advanced technologies and bioinformatics techniques to describe gene expression in stem cells during development, and in adult stem cells during tissue maintenance and tissue repair following disease. To build on these projects, a program is planned to link the GAPs with individual investigator-initiated projects.

A number of diseases are caused primarily by a single gene. Yet, disease symptoms and severity often differ between individuals, likely as a result of "modifier" genes whose activities may vary from person to person. Some of these modifier genes may exert broad control over basic cellular processes (these are often referred to as "housekeeping genes"). Others may be specific to the particular organ or tissue that is affected in the disease. The NIDDK is launching a research initiative, "Genetic Modifiers of Mendelian Diseases of Interest to NIDDK," in order to solicit research to identify genes that may influence the course of a number of diseases, including cystic fibrosis and hemochromatosis (discussed in later chapters). The hope is that by understanding modifying elements in different diseases, new therapeutic targets may be identified to mitigate the damage and suffering caused by a disease.

Through investment in these and other efforts, the NIDDK can continue to foster advances and opportunities by supporting basic research that will propel our understanding of and ability to treat disease.

DR. JAMES ROTHMAN

"Traffic Report"—Lasker Award Honors Scientists for Fundamental Discoveries About the Transport of Molecules Through Cells

One of the two winners of the 2002 Albert Lasker Award for Basic Medical Research is Dr. James E. Rothman. This award is very prestigious—many of its former recipients have subsequently won a Nobel Prize. Dr. Rothman is a long-time grantee of the National Institute of Diabetes and Digestive and Kidney Diseases, and he has also been supported by the National Cancer Institute (NCI) and the National Institute of General Medical Sciences (NIGMS). Now Chairman and Paul A. Marks Chair of the Cellular Biochemistry and Biophysics Program and Vice Chairman of the renowned Sloan-Kettering Institute, Dr. Rothman was honored for his discoveries of the machinery that regulates traffic in a cell. The exquisitely-organized trafficking of molecules within a cell and between a cell and its external surroundings underlies extraordinarily diverse biological processes. These include, for example, insulin secretion, neurotransmitter release, the transport of proteins that bring glucose and other nutrients into a cell, and the transport of proteins that serve as channels for salt ions to flow into or out of cells. Defects in the transport of proteins to their proper locations are associated with diseases such as cystic fibrosis and diabetes.

Different activities within a cell take place in specially-designated compartments, which, like the entire cell itself, are surrounded by membranes. Small membrane-enclosed shuttles called vesicles transport cargo from one cellular compartment to another, from within a cell to the cell surface membrane, and between a cell and its environment. Dr. Rothman's experiments unraveled the mystery of how vesicles form and transport their contents. His approach was to break open cells and mix various cellular materials back together to try to reconstitute membrane trafficking in a test tube. This would allow him to purify the components of this mixture down to the individual molecules necessary for trafficking and to discover their identity. While this type of approach had revealed clues to other cellular processes, at the time Dr. Rothman began his experiments, many scientists believed that protein trafficking would not work outside the orderly confines of a cell. It seemed unlikely that vesicles, set adrift in a test tube, would find the appropriate target membrane-bound compartments and deliver their cargo. But they did.

Dr. Rothman and his first postdoctoral fellow developed a clever way to detect this trafficking, using extracts from two different types of mammalian cells. One of these contained a viral protein called VSV G, but was deficient in an enzyme that normally attaches a particular sugar molecule onto the VSV G protein within an intracellular membrane-bound compartment. The other type of cell had this enzyme but not the viral protein. When the scientists mixed the two extracts, they found that the VSV G protein had acquired its sugar molecule. This indicated that the VSV G protein had been successfully transported—in a test tube—from a membrane-bound compartment of its original cell to one from the other type of cell, where the enzyme resided.

As they refined and varied their cell-free membrane trafficking system, Dr. Rothman and the members of his laboratory gained insight into how transport occurs. When cargo is to be sent out of a cellular compartment, it becomes wrapped in a stretch of membrane that then pinches off from the rest

DR. JAMES ROTHMAN

of the compartment to form a small, sealed, membrane-enclosed structure—the vesicle. This process is called "budding." Dr. Rothman's laboratory found that, as these vesicles form, they acquire a protein "coat." The coated vesicle then travels to its destination.

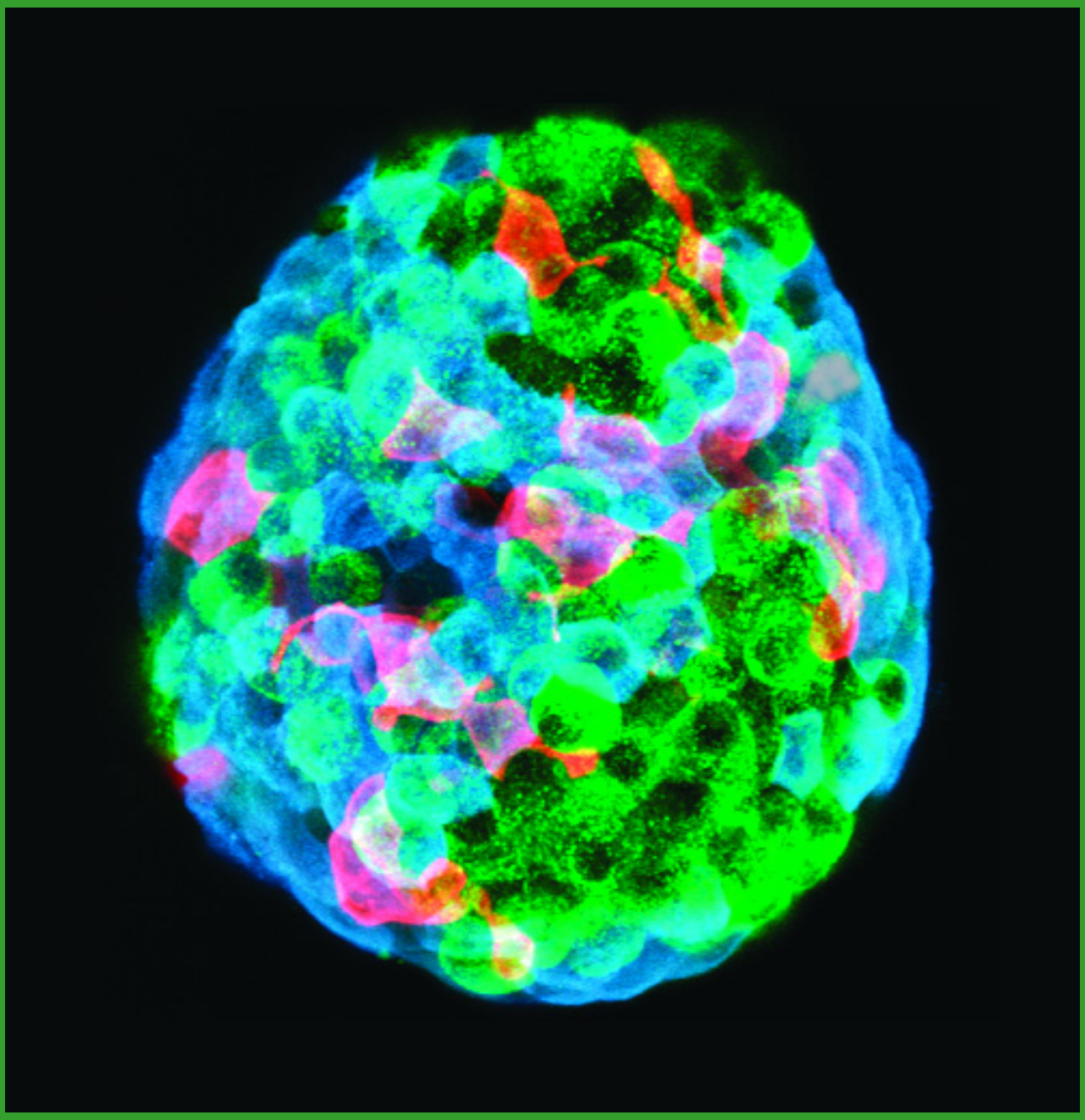
As a vesicle reaches its target location, it sheds its coat, and its membrane merges with the membrane of the target compartment in a process called "fusion." The stretch of membrane originally from the vesicle opens up to release its contents. In their investigation of membrane fusion, Dr. Rothman's laboratory identified proteins they called NSF and SNAP as important for this process. The discovery of NSF not only shed light on the fusion of membranes, but it also marked the converging of two different lines of inquiry into membrane trafficking. Working independently, another scientist, Dr. Randy W. Schekman, had also discovered NSF, but rather than reconstituting membrane transport from mammalian cell components in a test tube, Dr. Schekman's strategy was to analyze yeast cells harboring genetic mutations that disrupted membrane trafficking. The fact that these different experimental approaches led to the identification of the same protein validated the results. The subsequent findings from each laboratory built upon and enhanced the research of the other, and Dr. Schekman, an NIGMS grantee, was honored with a Lasker award along with Dr. Rothman.

Dr. Rothman's laboratory soon discovered another set of proteins integral to membrane fusion; they called these proteins SNAREs. SNAREs protruding from the membranes of vesicles (v-SNAREs) latch onto SNAREs residing in the target membranes (t-SNAREs) to form "SNAREpins." This interaction initiates fusion between the vesicle and target membranes. SNAREs also confer specificity on this process. It is critical that each of the many vesicles traveling about a cell dock at the correct target destination, because unloading cargo in the wrong place could wreak havoc in the cell. Dr. Rothman's laboratory demonstrated that, among the variety of different v-SNAREs and t-SNAREs, only certain combinations interact productively. Thus, a vesicle carrying a particular v-SNARE will only fuse with a target membrane that displays the appropriate t-SNARE. Once the membranes have fused, the earlier-identified proteins NSF and SNAP break apart the SNARE complex so that the SNARE proteins can be recycled.

Dr. Rothman's research continues to shed light on different aspects of cellular trafficking. Additionally, many scientists are investigating the association between defects in this process and disease. For example, insulin normally stimulates vesicle-mediated translocation of a protein called GLUT4 from the inside of cells to the cell surface membrane, where it functions to transport glucose into the cells. A defect in the insulin-induced trafficking of GLUT4 contributes to insulin resistance in muscle and fat cells in type 2 diabetes. The disease cystic fibrosis most commonly results from a mutant CFTR protein that cannot be exported to its proper location in the cell surface membrane. Left inside the cell instead, it does not function properly and is ultimately destroyed. One of the many strategies scientists are investigating towards treating cystic fibrosis involves tweaking the system that retains CFTR in an intracellular compartment so that the mutant CFTR proteins can escape to the cell surface membrane. Future research on vesicle transport will undoubtedly provide new insights into human disease.

For additional information on Dr. Rothman's research, see:

1. Rothman JE. Commentary—The machinery and principles of vesicle transport in the cell. *Nat Med* 8(10): 1059-1062, 2002, and references within.
2. The Lasker Foundation, http://www.laskerfoundation.org/awards/library/2002basic.html



An islet—Islets are clusters of cells within the pancreas containing the insulin-producing beta cells critically important in diabetes. This islet is labeled to show beta cells, which produce insulin, in green; alpha cells, which produce glucagon, in blue; and delta cells, which produce somatostatin, in red. Photo credit: Dr. Todd C. Brelje and Dr. Robert L. Sorenson, Islet Biology Laboratory, Department of Genetics, Cell Biology and Development, University of Minnesota.

Diabetes, Endocrinology and Metabolic Diseases

Chronic diseases affect tens of millions of Americans. Many of these diseases are caused by perturbations of the metabolic and endocrine (hormone) pathways that control energy balance and cellular functions. NIDDK-supported scientists are pursuing basic and clinical research on endocrinology, including osteoporosis, and metabolic diseases, including cystic fibrosis and obesity. At the intersection of these two fields is diabetes mellitus, a debilitating illness that is triggered by loss of activity of an endocrine hormone, with dire metabolic consequences.

Diabetes mellitus affects an estimated 17 million people in the U.S. and is the sixth leading cause of death. The disease lowers average life expectancy by up to 15 years, increases cardiovascular disease risk two- to four-fold, and is the main cause of kidney failure, lower limb amputations, and adult-onset blindness. Diabetes is marked by deficiencies in the body's ability to produce and properly use insulin—a hormone that is essential for the conversion of food-derived glucose into energy necessary for daily life. As a result, glucose becomes elevated in the blood, with detrimental effects throughout the body. The most common forms of the disease are type 1 diabetes, in which insulin-producing capacity is totally destroyed, and type 2 diabetes, in which the body is resistant to insulin, even though some amount of insulin may be produced.

Type 1 diabetes most often occurs in children, but may appear at any age and affects about 5 to 10 percent of people with diagnosed diabetes. Type 1 diabetes develops when the body's system for fighting infection—the immune system—turns against itself in a disease process called "autoimmunity." The immune system destroys clusters of cells in the pancreas called islets, which contain the body's insulin-producing beta cells. Once these cells are destroyed, type 1 diabetes patients require either lifelong insulin injections, multiple times every day, or infusion of insulin *via* a pump to control their blood glucose levels. Insulin therapy, however, is not a cure, nor can it always prevent the long-term complications of the disease.

Type 2 diabetes accounts for up to 95 percent of diabetes cases and affects about 8 percent of the U.S. population aged 18 and older. It is strongly associated with obesity; more than 80 percent of people with type 2 diabetes are overweight or obese. Type 2 diabetes is also associated with aging, affecting 20 percent of Americans over 65 years of age. It occurs more frequently among minority groups, including African Americans, Hispanic Americans, Native Americans, and Native Hawaiians. In patients with type 2 diabetes, cells in muscle, fat, and liver tissue do not respond effectively to insulin. Gradually, the pancreas secretes less and less insulin in response to meals, and the timing of insulin secretion becomes abnormal. As clinically recognizable diabetes develops, production of insulin continues to decline. To control glucose levels, treatment approaches include diet, exercise and medications; some patients also need to take insulin.

Rates of diabetes are expected to rise substantially as the U.S. population becomes increasingly overweight, sedentary, and racially and ethnically diverse. In addition to the burden on those affected and their families, the projected increase in diabetes will

also have major consequences for health care costs and the economy. In addition to the estimated 17 million Americans who have diabetes, another 16 million have "pre-diabetes," in which blood glucose levels are higher than normal but not yet as high as in diabetes. Pre-diabetes is itself associated with an increased risk of cardiovascular disease and with a high rate of progression to diabetes over a 5-to-10 year interval. Yet, the results of the Diabetes Prevention Program clinical trial show us that we can dramatically reduce the development of type 2 diabetes in those at highest risk through improvements in lifestyle or with medication.

Especially alarming are the increasing reports of type 2 diabetes in children and adolescents. This disease, once found almost exclusively in adults, is now affecting the next generation of Americans and is disproportionately affecting minority youth. These reports are of concern for several reasons. First, the onset and severity of 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 the offspring. Thus, the rising rates of diabetes and pre-diabetes in young women could lead to a vicious cycle of ever-growing rates of diabetes. Third, diabetes often becomes more difficult to control over time. With longer duration of disease, health care providers may find it increasingly difficult to strictly control a patient's blood sugar and thus prevent or delay the development of complications. Moreover, if current trends continue unabated, we may be seeing just the tip of the iceberg with respect to the future public health burden of diabetes on our society.

The NIDDK leads a vigorous research agenda throughout the NIH, designed to maximize our prospects of preventing, more effectively treating, and curing diabetes, as we move forward.

BLOOD SUGAR CONTROL IN TYPE 1 DIABETES: A BALANCING ACT

Cells in the body fuel their many complex functions by metabolizing the simple sugar glucose. But the body can suffer from too much of a good thing. In order to absorb the glucose from the bloodstream, especially after a meal, cells need to receive a signal. This signal is the hormone insulin. When insulin secretion is lost, as in type 1 diabetes, or cells no longer respond effectively to insulin, as in type 2 diabetes, blood sugar levels rise dramatically.

In recent reports, researchers offer further insights into the benefits and potential risks of blood sugar control first examined in the Diabetes Control and Complications Trial (DCCT). This large, randomized, and controlled clinical trial, completed in 1993, definitively tested the hypothesis that improved blood sugar control prevents or delays complications in diabetes. Type 1 diabetes patients in the DCCT trial were treated with either intensive or conventional therapy to control their blood sugar levels. Both groups were tested on a regular basis to identify onset or progression of complications. The researchers found that intensive control of blood sugar dramatically reduced the incidence of eye, nerve, and kidney disease, as compared to conventional therapy. The risk of developing these complications was directly related to the patient's average blood sugar during the period of the trial. The positive effects of intensive treatment were so conclusive that the trial was halted earlier than planned, at 6.5 years, and patients in the conventional treatment group were encouraged to change to intensive treatment during a closeout phase. As a result of this landmark trial, patients and health care providers were given concrete evidence that they could prevent or slow diabetic complications by carefully controlling blood sugar levels.

Continued Benefits of Intensive Blood Sugar Control: In a report on a follow-up study to the DCCT, scientists demonstrated that the benefits of strict blood sugar control first observed in the DCCT persist for at least 7 years. When the DCCT trial ended, both groups were encouraged to use intensive therapy and were closely monitored. Overall blood sugar levels soon became similar in the two groups—with better control in the former conventional group and looser control in the former intensive group. Despite their similar levels of control during the subsequent 7 years, follow-up showed that those who had initially been in the intensive therapy group were still less likely to develop eye or kidney disease than those whose blood sugar was initially controlled using conventional therapy.

This follow-up study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, is still ongoing. Using the DCCT closeout examination results as a baseline for comparison, the scientists found that for patients initially in the intensive treatment group, progression of retinopathy (eye disease) was reduced between 66 and 77 percent, and the risk for progression or development of decreased kidney function, as indicated by the presence of protein in the urine, was decreased by 84 percent. Prevalence of high blood pressure, an almost inevitable consequence of reduced kidney function, also differed between the former intensive and conventional DCCT treatment groups. Six years after the beginning of EDIC, 33 percent of those who had initially practiced conventional blood sugar control developed high blood pressure, as compared to only 25 percent of those who had practiced intensive control.

The DCCT results demonstrated that by strictly monitoring and controlling their blood sugar, patients can significantly reduce their risks of developing complications. The new results from EDIC show that the benefits of a finite period of stricter blood sugar control persist for at least 7 years after levels of control were equalized. Because of this proven, persistent benefit, it is important to begin intensive treatment to control blood sugar as early as possible.

Potential Risks of Overly Tight Blood Sugar Control: Somewhat clouding the long-term benefits of strict blood sugar control demonstrated by the DCCT and EDIC are the short-term risks patients face. Episodes of dangerously low blood sugar—hypoglycemia—are a common, acute complication of type 1 diabetes and, at least in the patients over 13 years of age studied in the DCCT, are increased by tight glucose control. Symptoms of hypoglycemia can range from mild to life-threatening. Awareness of risk factors for this condition and accurate measurement of blood glucose levels are thus extremely important for the management of type 1 diabetes.

NIDDK-supported investigators recently studied 415 children, adolescents, and young adults with type 1 diabetes to define risk factors for frequent and severe hypoglycemia in this population. Frequent hypoglycemia—defined as two or more episodes per week—occurred in a third of the study participants and was associated with better blood glucose control, intensive insulin therapy, and frequent self-monitoring of blood glucose. Severe hypoglycemic events leading to loss of consciousness were reported by only 4-to-7 percent of the participants. These severe episodes were correlated with better blood glucose control and older age. Notably, a history of frequent hypoglycemia did not predict who would experience severe episodes. These research findings will help type 1 diabetes patients and their medical care providers to better manage this disease by becoming aware of the risk factors for hypoglycemia, while still maintaining optimal glucose control for the prevention of diabetic complications.

Emerging Technologies for Measuring Blood Sugar: Non-invasive or minimally invasive glucose monitoring systems have the potential to improve glucose control and minimize the risk of hypoglycemia. A new continuous monitoring system that samples glucose in body fluid without the need to puncture the skin and draw blood may make management of diabetes more convenient. NIDDK-supported researchers examined how accurately this system reflects blood glucose levels during periods of hypoglycemia or hyperinsulinemia (extremely high insulin). In non-diabetic volunteers, the sensor

accurately measured glucose levels and detected periods of hypoglycemia, provided that the sensor was first calibrated across a wide range of glucose and insulin levels—thus bringing this technology one step closer to widespread use.

Type 1 diabetes requires a continual balancing of glucose and insulin within normal ranges, not only to sustain life, but also to minimize the devastating complications caused by the long-term elevation of blood glucose. Patients with type 2 diabetes face similar risks of complications from consistently elevated blood sugar. If doctors and patients adopt stricter standards of blood sugar monitoring and control that are well-informed by data on the risks of overly intensive blood sugar control, it is likely that we can prevent or delay the development of long-term complications in the estimated 17 million American with diabetes, both type 1 and type 2, and minimize the risk of hypoglycemia.

Allen C, LeCaire T, Palta M, Daniels K, Meredith M and D'Alessio DJ: Risk factors for frequent and severe hypoglycemia in type 1 diabetes. *Diabetes Care* 24: 1878-81, 2001.

Monsod TP, Flanagan DE, Rife F, Saenz R, Caprio S, Sherwin RS and Tamborlane WV: Do sensor glucose levels accurately predict plasma glucose concentrations during hypoglycemia and hyperinsulinemia? *Diabetes Care* 25: 889-93, 2002.

The Writing Team for the Diabetes Control and Complications Trial: Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 287: 2563-9, 2002.

INJECTED INSULIN DOES NOT DELAY ONSET OF TYPE 1 DIABETES

Type 1 diabetes is also known as insulin-dependent diabetes because it forces a patient to depend upon daily, external insulin administration to stay alive. When patients are first diagnosed with type 1 diabetes, their beta cells still produce some residual amount of insulin. Subsequently, however, most patients gradually lose the ability to produce insulin. Based on animal data and very small pilot studies in humans, many patients and health care providers hoped and believed that administering very low dose injections of insulin to those at highest risk of developing the disease might actually prevent onset of diabetes, either by allowing the struggling beta cells to rest or by altering the immune system by an as yet unknown mechanism. In fact, relying upon these early reports, some doctors began treating high risk patients with insulin, even though its usefulness in preventing type 1 diabetes had not been adequately tested.

In a major clinical trial, NIDDK-supported researchers recently tested the hypothesis that injected insulin can prevent type 1 diabetes in those most at risk—in this case, relatives of type 1 diabetes patients. Genetic tests and measurement of antibodies directed against components of the insulin-producing beta cells, combined with assessment of insulin secreting capacity through metabolic testing, can be used to screen these individuals and predict risk for the development of type 1 diabetes. In designing this study, the researchers believed that they could accurately identify a very high-risk group of relatives, half of whom were likely to develop type 1 diabetes over the course of 5 years.

In the trial, relatives of type 1 diabetes patients with anti-islet antibodies and other genetic, metabolic, and immunologic indicators of susceptibility to the disease were assigned to either an intervention or an observational group. Those in the former group received low dose injections of slow-acting insulin twice every day, in addition to yearly, 4-day continuous intravenous insulin infusions. Participants were tested every 6 months to determine whether or not their bodies were able to effectively manage blood sugar.

After an average follow-up of 3.7 years, there was no significant difference in the number of participants who developed type 1 diabetes in the insulin-injection group versus the observational group,

demonstrating that the injected insulin therapy was not effective in preventing disease onset. Notably, those in the insulin treatment group did not develop low blood sugar or any other side effects—a concern when administering insulin.

This trial unequivocally disproved the hypothesis that injected insulin can prevent type 1 diabetes in those at risk, thus potentially sparing many patients from burdensome and ineffective therapy. It also clearly demonstrated that researchers can predict, with great reliability, which relatives of type 1 diabetes patients are most likely to develop the disease, using methods and knowledge derived from years of carefully conducted research studies. This ability is extremely important because it will allow researchers to design studies to test promising new preventive agents as they are developed or identified.

This study was part of the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1). A parallel trial, testing the efficacy of orally administered insulin for preventing the onset of type 1 diabetes in persons at increased risk (25-to-50 percent risk of developing disease in 5 years), is still ongoing. This study is being conducted through TrialNet, a large consortium of 14 clinical centers, a data coordinating center, and laboratory facilities in the U.S. and Canada established by the NIDDK to conduct rapid, preliminary clinical trials for therapies that may delay, reverse, or prevent type 1 diabetes. As information and biological samples are collected from at-risk and newly-diagnosed patients at the various clinical sites, TrialNet will also provide an invaluable resource to researchers interested in identifying genes that contribute to susceptibility to type 1 diabetes and its complications–further leveraging the NIDDK's investment in this important clinical initiative.

Diabetes Prevention Trial-Type 1 Diabetes Study Group: Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med* 346: 1685-91, 2002.

FACING DOWN AUTOIMMUNITY IN TYPE 1 DIABETES PREVENTION AND TREATMENT

In type 1 diabetes, certain cells of the immune system, T cells, mistakenly attack and destroy the insulin-producing beta cells of the pancreas. Though the precise genetic and environmental factors that trigger this autoimmune process are still being elucidated, scientists can now identify individuals who are at high risk of developing type 1 diabetes (see previous section). Thus, a major goal of type 1 diabetes research is to find ways to block the autoimmune attack leading to diabetes in at-risk persons or to arrest or reverse this process in new-onset patients. Ideally, methods aimed at arresting diabetes-related autoimmunity should avoid causing global suppression of the immune system.

Researchers recently used mouse models of type 1 diabetes to gain new insights into the cellular mechanisms that are capable of suppressing the diabetic autoimmune process. In a clever study aimed at manipulating the immune system, NIDDK-supported investigators tested an engineered molecule, called "DEF." DEF is a soluble molecule which consists of a specific peptide (a small piece of protein) linked to a major histocompatibility complex (MHC) molecule. MHC molecules are normally present on body cells and function both to alert the immune system to infection by "presenting" pieces of microbe proteins (antigens) to immune system cells, and also to enable the immune system to distinguish "self" from "non-self." When T cells bind to MHC-protein complexes on other cells, the interaction can stimulate or repress T cell activities.

The researchers found that, in their mouse model, the soluble DEF molecule was able to prevent the development of disease in pre-diabetic animals and to reverse it in mice that had recently developed diabetes. DEF was shown to shut off a group of T cells in the spleen that would otherwise migrate to the pancreas and attack the pancreatic beta cells. In addition, DEF turned on a different set of T cells located in the pancreas that seemed to protect the beta cells from autoimmune attack.

In a similar study, an antibody targeting CD40L, a protein that helps "switch on" autoimmune T cells, prevented type 1 diabetes in a second mouse model of this disease. Protection from diabetes was conferred by a previously-undescribed cell population, composed of dendritic and natural killer cells, that muted the autoimmune response. Importantly, both immune system modulating agents—DEF and the CD40L antibody—specifically repressed autoimmunity in susceptible mice without inducing global immune suppression.

Moving from mouse models to potential human therapeutics, a recent small-scale clinical trial conducted by NIDDK-supported researchers has provided a glimmer of hope for those newly-diagnosed with type 1 diabetes. Twelve patients diagnosed within the previous 6 weeks were injected with a modified form of an antibody known as anti-CD3. This antibody works by suppressing the immune system's destructive T cells and by stimulating the production of protective immune-signaling molecules. Twelve other patients received no anti-CD3 injections and served as a control group. Nine of the 12 treated patients maintained or improved their ability to produce insulin for 1 year following diagnosis. In contrast, all but two of the 12 untreated patients in the control group experienced a decline in insulin production.

Preservation of beta cell function is important because those patients with diabetes who can still make some insulin are able to achieve better control of blood sugar and have less risk of low blood sugar reactions than patients with little or no ability to produce insulin. This encouraging but very preliminary finding with anti-CD3 will now be tested in larger numbers of patients in a study sponsored by the Immune Tolerance Network (ITN), an NIH research effort spearheaded by the National Institute of Allergy and Infectious Diseases (NIAID) with support from the NIDDK and the Juvenile Diabetes Research Foundation. The purpose of the ITN is to accelerate the development of new tolerance therapies to treat human conditions, including transplant rejection, autoimmunity and asthma, and allergic diseases. If it proves effective in new-onset diabetes patients in larger trials, anti-CD3 treatment will then be studied in individuals at high risk for type 1 diabetes to determine whether it can actually prevent development of the disease.

Together, these findings represent an important step forward in our understanding of how to selectively halt or reverse the autoimmune process at the heart of type 1 diabetes. The results of these studies reveal new cellular targets that researchers can home in on as they search for novel therapies to prevent or reverse this disease. Understanding the mechanism through which these agents work will help researchers to optimize the design and analysis of immuno-prevention trials in human patients.

Casares S, Hurtado A, McEvoy RC, Sarukhan A, von Boehmer H and Brumeanu TD: Down-regulation of diabetogenic CD4+ T cells by a soluble dimeric peptide-MHC class II chimera. *Nat Immunol* 3: 383-91, 2002.

Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, and Bluestone JA: Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med* 346: 1692-98.

Homann D, Jahreis A, Wolfe T, Hughes A, Coon B, van Stipdonk MJB, Prilliman KR, Schoenberger SP and von Herrath MG: CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. *Immunity* 16: 403-15, 2002.

CARDIOVASCULAR DISEASE AND DIABETES

Cardiovascular disease (CVD) is the leading cause of death in individuals with diabetes. Among patients with type 2 diabetes, many already have increased CVD risk factors, such as elevated cholesterol and blood pressure, at the time they are diagnosed with the disease.

NIDDK-supported researchers recently completed a survey of the health status of over 117,000 women, who were followed for 20 years as part of the Nurses' Health Study and had not been diagnosed with CVD prior to the study. Compared with non-diabetic women of similar age, those who developed type 2

diabetes during the study period had a significantly increased risk of heart attack and stroke, even when adjusted for other CVD risk factors. Strikingly, this risk began to rise at least 15 years before a diagnosis of type 2 diabetes. Thus, persons who are at risk for type 2 diabetes or who have been diagnosed with "pre-diabetes" should be aware of and aggressively manage their underlying cardiovascular risk factors.

Relevant to prevention of CVD in patients with diabetes is a recently completed clinical trial in patients with coronary disease, low blood levels of high-density lipoprotein (HDL—the "good cholesterol"), and normal levels of low-density lipoprotein (LDL—the "bad cholesterol"). This lipid profile is commonly seen in diabetes. The study found that the combination of two lipid-lowering statin drugs, simvastatin and niacin, provides significant benefit in these patients. By contrast, antioxidant vitamins—vitamin E, vitamin C, and beta carotene—as well as selenium, were not effective. NIDDK-supported researchers observed that the patients' coronary disease (the degree of blood vessel narrowing) showed slight regression after 3 years on the simvastatin-niacin combination, while the incidence of major clinical events—primarily heart attack and stroke—was reduced 90 percent. However, antioxidant vitamins, which previously were thought to possibly provide cardiovascular protection, had no such effect. Moreover, when antioxidants were added to the simvastatin-niacin therapy, the clinical benefits were diminished as compared with those achieved with simvastatin and niacin alone. The results seen with simvastatin-niacin therapy, if confirmed, could represent a major advance in the prevention of heart attack and stroke in patients with coronary disease and a low HDL/normal LDL profile. It also appears that there is little justification for antioxidant therapy for cardiovascular disease in this patient population at this time.

Stressing the importance of CVD risk factor assessment and management is at the core of the "Be Smart About Your Heart—Control the ABCs of Diabetes" campaign currently being run by the National Diabetes Education Program, a joint effort of the NIDDK and the Centers for Disease Control and Prevention (CDC) (see sidebar, "Diabetes Education at NIDDK—The National Diabetes Education Program"). This campaign is designed to make people with diabetes aware of their high risk for heart disease and stroke and the steps they can take to dramatically lower that risk. The campaign emphasizes managing blood glucose (best measured by the A1C test), blood pressure, and cholesterol.

Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J and Albers JJ: Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 345: 1583-92, 2001.

Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC, and Manson JE: Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care* 25: 1129-34, 2002.

NEW INSIGHTS INTO HOW THE *CALPAIN-10* GENE AFFECTS RISK FOR TYPE 2 DIABETES

Both genetic and environmental factors contribute to the risk of both type 1 and type 2 diabetes. The genetic contribution is likely due to multiple "susceptibility genes," each of which modestly increases risk. Adding to the complexity, different genetic factors may play a role in different populations. This is because humans share a standard set of genes, but there is much variation, or polymorphism, within our genes, which can affect how well they are expressed or how well the gene products function or interact with each other. This, in turn, can translate into critical alterations in the metabolic pathways and immune system interactions involved in diseases such as diabetes. Different racial and ethnic groups often possess distinct sets of gene polymorphisms that interact to confer susceptibility to diabetes.

Previously, NIDDK-supported researchers identified a gene, *calpain-10*, associated with an increased risk of type 2 diabetes in Mexican Americans. This gene was subsequently shown to confer an increased risk of diabetes in several, but not all, Northern European populations studied and in the Pima

Indians, who have the highest rates of diabetes in the world. *Calpain-10* is a member of a calcium-activated neutral protease family. The protein made by the *calpain-10* gene is found in pancreatic islets, muscle, and liver—the three key tissues that control blood glucose levels. Thus, *calpain-10* might regulate pathways that affect insulin secretion, insulin action, and glucose production by the liver, each of which is altered in type 2 diabetes. Recent studies have shed more light on both the effect of *calpain-10* polymorphisms in different populations and on how *calpain-10* contributes to diabetes risk.

African Americans are at increased risk for type 2 diabetes, but relatively little is known about the genes that may contribute to this risk. NIDDK-supported researchers studied the link between the genetic changes in the *calpain-10* gene and the risk of having or subsequently developing type 2 diabetes in a large group of middle-aged African Americans participating in the Atherosclerosis Risk in Communities Study (ARIC, a study supported by the National Heart, Lung, and Blood Institute). They found that, as was seen in the Mexican Americans, individuals with a specific genetic variation of the *calpain-10* gene have a moderately increased risk of developing type 2 diabetes. The high frequency of this genetic polymorphism of *calpain-10* in African Americans suggests that it may account for as much as 25 percent of the risk of diabetes in this population.

Although alterations in the calpain gene have not been strongly linked to diabetes in British individuals, researchers decided to study the effect of the specific genetic changes or polymorphisms in the *calpain-10* gene that have been associated with increased risk of diabetes in other populations in 285 people without diabetes in Britain. They found that these variations in the *calpain-10* gene were associated with a small impairment of the early, rapid secretion of insulin in response to eating, leading to elevated blood glucose levels. Individuals with these alterations in the *calpain-10* gene were also less responsive to the action of insulin.

How might the *calpain-10* gene exert its effects on diabetes susceptibility? In pursuing the mechanism by which alterations in *calpain-10* might cause diabetes, NIDDK-supported researchers discovered that the genetic variations in the *calpain-10* gene that have been associated with increased diabetes risk result in reduced levels of calpain-10 protein in human skeletal muscle. This is important because muscle is the major site of glucose uptake in response to insulin. Researchers then applied inhibitors of calpain protein activity to mouse muscle and fat tissue to investigate the biochemical pathways that may be regulated by this gene product. Calpain inhibition reduced glucose uptake into fat and muscle in response to insulin and reduced synthesis of glycogen, which is made from glucose to store energy in muscle. These results confirm and extend earlier findings in non-diabetic humans, in which low levels of calpain-10 in the muscle were associated with insulin resistance.

Researchers also studied the effect of calpain inhibitors on mouse pancreatic islets, the site of insulin-producing beta cells; the calpain inhibitors enhanced glucose-induced insulin secretion, suggesting that calpain may also play a role in regulating insulin secretion in the pancreatic islets.

Identifying the genes predisposing to a disease is important because understanding how genetic changes cause disease can help researchers identify pathways that may be useful in treating or preventing disease. These findings suggest a role for calpains in the regulation of insulin secretion and insulin action and extend the populations in which genetic variations in the *calpain-10* gene appear to contribute to the risk of developing type 2 diabetes. Further studies are needed to more precisely identify the mechanisms underlying the association of alterations in *calpain-10* with diabetes and altered glucose metabolism.

Calpain 10 is one of several susceptibility genes under investigation for its role in diabetes, and researchers expect to find many more. The NIDDK is vigorously supporting the search for type 1 and type 2 diabetes susceptibility genes through two major genetics consortia. These collaborative and international efforts include the Type 1 Diabetes Genetics Consortium, which is striving to identify type 1 diabetes susceptibility genes by "scanning" human genome sequences in families from the U.S., Europe, and Australia; and the International Type 2 Diabetes Genetic Linkage Analysis Consortium, which has recently enhanced its data set with more samples from African Americans, who are at disproportionately high risk for type 2 diabetes. By pooling data collected from thousands of individuals through these consortia, investigators can more rapidly perform statistically meaningful analyses that will enable them to identify other susceptibility genes that contribute to diabetes.

Garant MJ, Kao WH, Brancati F, Coresh J, Rami TM, Hanis CL, Boerwinkle E, and Shuldiner AR: SNP43 of *CAPN10* and the risk of type 2 diabetes in African-Americans: The Atherosclerosis Risk in Communities Study. *Diabetes* 51: 231-7, 2002.

Lynn S, Evans JC, White C, Frayling TM, Hattersley AT, Turnbull DM, Horikawa Y, Cox NJ, Bell GI, and Walker M: Variation in the *calpain-10* gene affects blood glucose levels in the British population. *Diabetes* 51: 247-50, 2002.

Sreenan SK, Zhou Y-P, Otani K, Hansen PA, Currie KPM, Pan C-Y, Lee J-P, Ostrega DM, Pugh W, Horikawa Y, Cox NJ, Hanis CL, Burant CF, Fox AP, Bell GI, and Polonsky KS: Calpains play a role in insulin secretion and action. *Diabetes* 50: 2013-20, 2001.

GENES DIRECTING PANCREATIC DEVELOPMENT

The pancreas has many critical functions in the body, including insulin production. So, just how does an insulin-producing beta cell become a beta cell? To create the specialized cells of the pancreas, precursor cells during embryonic development begin to activate select genes that will equip their progeny cells with the necessary tools to become pancreatic cells—including insulin-producing beta cells. Scientists are continuing to identify the genes essential to pancreatic development.

NIDDK-supported scientists recently discovered in mice that a gene called *Ptf1a* is critical for directing cells to become pancreatic cells. *Ptf1a* encodes a protein important for regulating the expression of other pancreas-specific genes. Using special genetically-engineered mice, the researchers found that *Ptf1a* was expressed in progenitor cells that developed into all three major types of pancreatic tissue; previously, it had been thought that *Ptf1a* was only important to the development of one pancreatic cell type. Furthermore, prospective pancreas cells deficient in *Ptf1a* expression instead became incorporated into the small intestine. This research thus underscores the importance of *Ptf1a* in determining cell fate and committing progenitor cells to form the pancreas.

Another gene, called *Pdx1*, has been found to play a role both in pancreatic development and in maintaining the function of the mature pancreatic beta cells that secrete insulin to control blood sugar levels. The *Pdx1*-encoded protein is another regulator of gene expression, like *Ptf1a*. Scientists already knew that *Pdx1* expression is necessary for initial pancreas development, but wanted to learn more about its role in later developmental stages. To do this, NIDDK-supported researchers used cleverly designed genetic engineering to create mouse strains in which they could suppress *Pdx1* expression at any point during development and adulthood by administering a particular drug. They found that by repressing *Pdx1* expression in mouse embryos either early or late in gestation, they could selectively prevent or halt pancreas development. The researchers also found that they could induce glucose intolerance and lower insulin production in adult mice by reducing *Pdx1* expression—confirming its importance in the molecular pathways regulating blood sugar. These results provide further proof of the importance of the *Pdx1* gene in both pancreatic development and

maintenance. Additionally, the researchers have also shown that controlling the expression of a critical regulatory protein is a valid and potentially quite useful approach for studying the formation or maintenance of organs in mice. This approach will likely be useful in future studies of other genes regulating pancreas development.

Holland AM, Hale MA, Kagami H, Hammer RE, and MacDonald RJ: Experimental control of pancreatic development and maintenance. *Proc Natl Acad Sci USA* 99: 12236-41, 2002.

Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, and Wright CVE: The role of the transcriptional regulator *Ptf1a* in converting intestinal to pancreatic progenitors. *Nat Genet* 32: 128-34, 2002.

TECHNOLOGY IN DIABETES RESEARCH

Advances in research are greatly accelerated by advances in or new uses of technology. Gene microarray technology (GMT) has transformed the collection of normal gene expression data in the past 5 years. GMT has also enabled researchers to obtain "snapshots" of global changes in cellular gene expression caused by disease.

In GMT, single strands of DNA taken from different genes are arrayed on a small glass slide in an organized grid, like a chessboard; the researcher has the master plan of where all the different pieces are on the board. To start an experiment, cellular RNA molecules—the molecules cells use to "express" the information encoded in genes—are chemically labeled for later detection and then applied to the grid; researchers can also synthesize and use so-called "cDNA" molecules, which are the DNA equivalent of these RNAs. When a labeled molecule finds a matching piece—i.e., its matching gene—on the board, it sticks to it. The increases or decreases in gene expression in cells grown under different conditions can then be measured: the more of one particular labeled RNA or cDNA there is in the applied solution, the more of that one will stick to the array.

However, the usefulness of GMT to diabetes research has been limited because most available chips are not specifically designed to contain genes expressed in tissues—most importantly, the pancreatic islets—or in molecular pathways involved in the pathogenesis of diabetes. Researchers have now assembled a set of 3,400 clones—discrete pieces of DNA—representing genes expressed in the pancreas or known to be relevant to diabetes. This clone set was used to construct the "PancChip," a microarray that can provide pancreas-specific gene-profiling data. The pancreas clone set is available to the diabetes research community through NIDDK-supported biotechnology centers. This resource will help scientists characterize the pancreatic beta cells that are central to diabetes.

Scientists have also used GMT to examine the profound changes in cellular gene expression, glucose and lipid metabolism, and protein phosphorylation (an important chemical modification) caused by deficiencies in insulin signaling in diabetes. The extent of these changes—and whether they can be reversed—is still being characterized. An NIDDK-supported research team recently used GMT to identify differences in gene expression in the skeletal muscle of normal and insulin-deficient, diabetic mice. Skeletal muscle is the major site of insulin-dependent glucose uptake in the body, and thus is of key importance for understanding healthy mechanisms of glucose disposal and how these are altered in diabetes.

In this study, 235 genes showed altered expression in skeletal muscle of diabetic animals. These 235 genes encode enzymes, transporters, and other molecules involved in glucose and lipid metabolism, protein degradation, and protein trafficking. Short-term insulin therapy in the diabetic mice restored the normal pattern of expression to only about half of the 235 genes, indicating that there is a lasting effect of the diabetic state (specifically, one induced by the destruction of pancreatic beta cells) on skeletal muscle gene expression.

Collectively, the altered expression patterns of many genes were consistent with both the decreased glucose usage and increased dependence upon fat burning that occur in the skeletal muscle of diabetic animals. Scientists can now use this new knowledge to help them identify shared gene-regulatory factors that may coordinate the insulin-dependent response of various metabolic pathways in the cell. Such shared factors might represent novel therapeutic targets for diabetes.

New technologies can also be crucial for swiftly evaluating the efficacy of disease therapy. For example, at present, the only reliable method for determining the number of beta cells in a patient with type 1 diabetes is to measure beta cell mass in a post-mortem examination. Although researchers are trying to develop methods to preserve as many beta cells as possible in patients newly diagnosed with type 1 diabetes and in those in which the autoimmune process has begun but not yet produced diabetes, their efforts are hampered by an inability to accurately determine the number of beta cells present. Without such a measurement, it is difficult for researchers to determine whether the number of beta cells has remained the same or if it has decreased, and thus to determine whether or not the intended therapy has been successful.

Using diabetic mice, a group of investigators recently made great strides toward developing an accurate method for measuring the number of beta cells in a living animal. The method relies upon intravenous administration of a radioactive probe, joined to an antibody, which is designed to bind specifically to pancreatic beta cells. The scientists found that the signal intensity of the bound probe correlated directly with a post-mortem measurement of beta cell mass in both normal and diabetic mice. This study provides a critical step toward the development of a method for determining and sequentially assessing the number of beta cells present in people with type 1 diabetes or at high risk for developing it. Once they can assess the number of beta cells present in living patients, researchers will be able to evaluate response to therapeutic interventions, including methods to preserve beta cell function before it is completely destroyed, and gain new insight into the autoimmune process that destroys the beta cells.

Moore A, Bonner-Weir S, and Weissleder R: Noninvasive in vivo measurement of beta-cell mass in mouse model of diabetes. *Diabetes* 50: 2231-36, 2001.

Scearce LM, Brestelli JE, McWeeney SK, Lee CS, Mazzarelli J, Pinney DF, Pizarro A, Stoeckert CJ, Jr., Clifton SW, Permutt MA, Brown J, Melton DA, and Kaestner KH: Functional genomics of the endocrine pancreas: The pancreas clone set and PancChip, new resources for diabetes research. *Diabetes* 51: 1997-2004, 2002.

Yechoor VK, Patti ME, Saccone R and Kahn CR: Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice. *Proc Natl Acad Sci USA* 99: 10587-92, 2002.

DEVELOPMENT OF NEW TECHNOLOGIES FOR THE TREATMENT OF CYSTIC FIBROSIS

Cystic fibrosis (CF), a recessive genetic disease, affects approximately 30,000 Americans and is diagnosed in approximately 1 in 4,000 children born each year. The gene responsible for CF, *CFTR*, produces a protein normally found in the membranes of secretory cells of the airway, pancreas, sweat glands, salivary glands, intestines, and reproductive organs. Although about 900 mutations have been identified, the delta F508 (ΔF508) mutation is most common, accounting for 70 percent of affected CF genes.

Research teams are exploring different ways to overcome the harmful effects of the ΔF508 mutation. One avenue is to develop safe, effective methods for correcting the defect by replacing the abnormal gene, in whole or in part, by a normal copy. However, the low efficiency of correction at the DNA level has proven to be an obstacle for gene transfer to become a viable treatment for humans. An alternative approach attacks the genetic problem at the messenger RNA (mRNA) level. Messenger RNA is the key intermediate in the process the cell uses

to make a protein from the instructions encoded in the DNA. NIDDK-supported researchers recently used SMaRT (spliceosome mediated RNA trans-splicing) technology to replace the ΔF508 mutation at the mRNA level. Instead of incorporating the part of the CFTR mRNA with the mutation, cells were provided with a partial mRNA containing a normal sequence that could be spliced into the mature mRNA molecule. In laboratory studies, this technique provided a partial restoration of the level of CFTR protein in airway cells obtained from CF patients.

SMaRT offers several advantages over conventional gene therapy approaches because only cells that normally express the *CFTR* gene are targeted by this therapy. Also, since SMaRT only replaces the abnormal portion of the message, researchers can take advantage of gene delivery technologies that are unable to accommodate the entire *CFTR* gene.

Another therapeutic approach is to combat the effects of the ΔF508 mutation at the level of the CFTR protein. This mutation results in improper folding and retention of CFTR protein within the cell and inhibition of CFTR transport to the cell membrane, where it normally functions as a chloride channel to maintain the cellular electrolyte (salt) and fluid balance. Because the cellular concentration of calcium plays a role in the retention of abnormal CFTR within cells, depletion of cellular calcium stores may allow the misfolded ΔF508 CFTR protein to "escape" from within the cell and reach the cell membrane where it may function effectively and thereby correct the CF defect. Genetically engineered mice with ΔF508 CFTR treated with an inhaled calcium binding agent had decreased calcium concentrations in cells lining the airway, thus permitting the ΔF508 CFTR protein to be transported to the cell membrane where it functioned to increase chloride transport.

These results suggest that alternate approaches may be more effective in reversing CFTR abnormalities than conventional gene transfer. Development of non-toxic and effective small molecule therapies that can be delivered directly into the airway may allow production of a normal CFTR protein or improve function of an abnormal CFTR protein, and thus correct the defect in chloride transport seen in CF. Research to develop these technologies further may lead to new treatments for CF, as well as for other inherited genetic diseases.

Egan ME, Glöckner-Pagel J, Ambrose CA, Cahill PA, Pappoe L, Balamuth N, Cho E, Canny S, Wagner CA, Geibel J, and Caplan MJ: Calcium-pump inhibitors induce functional surface expression of ΔF508-CFTR protein in cystic fibrosis epithelial cells. *Nat Med* 8: 485-92, 2002.

Liu X, Jiang Q, Mansfield SG, Puttaraju M, Zhang Y, Zhou W, Cohn JA, Garcia-Blanco MA, Mitchell LG, and Engelhardt JF: Partial correction of endogenous ΔF508 CFTR in human cystic fibrosis airway epithelia by spliceosome-mediated RNA trans-splicing. *Nat Biotechnol* 20: 47-52, 2002.

GENE THERAPY IN DOGS WITH MUCOPOLYSACCHARIDOSIS VII (MPS VII)

Mucopolysaccharidosis VII (MPS VII) is an inherited disease characterized by heart and eye abnormalities, poor growth, mental retardation, mobility problems, liver and spleen enlargement, and other serious symptoms. It is caused by a deficiency in the enzyme beta-glucuronidase. Without beta-glucuronidase, cells cannot properly break down complex carbohydrate molecules called mucopolysaccharides. This results in the abnormal storage of mucopolysaccharides in various tissues, leading to the observed physical symptoms. Currently, no truly effective treatments for MPS VII are available.

NIDDK-supported researchers have now successfully tested a new strategy for MPS VII treatment in dogs with the disease—gene therapy. Based on earlier studies in mice, the scientists used a special genetically-engineered virus to insert a functional copy of the gene for beta-glucuronidase into liver cells of newborn dogs with MPS VII. Their hope was that the liver cells would manufacture the enzyme and release it into the bloodstream to be carried to other affected organs. The experimental treatment worked, preventing heart, eye and other symptoms that the scientists could assess in dogs. The scientists

will continue to monitor the animals for potential adverse effects of the gene therapy. This research may one day lead to gene therapy treatments not only for people with MPS VII, but also for those with other diseases caused by deficiencies in blood or liver proteins.

Ponder KP, Melniczek JR, Xu L, Weil MA, O'Malley TM, O'Donnell PA, Knox VW, Aguirre GD, Mazrier H, Ellinwood NM, Sleeper M, Maguire AM, Volk SW, Mango RL, Zweigle J, Wolfe JH, and Haskins ME: Therapeutic neonatal hepatic gene therapy in mucopolysaccharidosis VII dogs. *Proc Natl Acad Sci USA* 99: 13102-07, 2002.

FIGHTING DISEASE BY TURNING ON GENES—INSIGHTS FROM VITAMIN D AND GUGGUL TREE RESIN

Scientists have discovered novel links between molecules made in the body called bile acids and two compounds that appear to mitigate some of the ill effects of high cholesterol or high-fat diets. One of these compounds, well-known in the U.S., is vitamin D. The other, perhaps better known in India, is guggulsterone, a component of guggul tree resin. In humans, an extract of this resin lowers the "bad" form of cholesterol in the body, LDL cholesterol, as well as triglycerides, and it received regulatory approval for medicinal use in India in 1987; the resin itself has been used for health care in India for over 2,000 years.

To learn how guggulsterone lowers cholesterol, NIDDK-supported scientists investigated its effects on bile acid production. Bile acids are made in the liver and are used as the body's natural way to rid itself of toxic substances and excess fats, including cholesterol. However, once bile acid levels are high, they trigger a negative feedback loop which prompts the liver to dampen further bile acid production and, as an unfortunate consequence, decrease the elimination of cholesterol. This feedback loop is initiated when the bile acids activate a protein called FXR. This protein is a steroid receptor that works by telling cells what genes to turn on or off. The bile-acid-activated FXR receptor turns on genes that work to reduce bile acid production. The scientists found that guggulsterone blocked the activation of FXR by bile acids in human cells grown in the laboratory, and that guggulsterone reduced cholesterol levels in normal mice fed a high-cholesterol diet. They concluded that guggulsterone lowers cholesterol by inhibiting FXR, so that even when bile acid levels are high, the liver can continue producing bile acids as a means of eliminating cholesterol.

In related work, researchers are gaining new insights about why some bile acids are quite harmful and how those harms might be mitigated. For example, increased levels of the most toxic of these, a bile acid called LCA, are associated with a high-fat diet, and accumulation of LCA is linked to liver damage and colon cancer. Working with isolated proteins and human cells, NIDDK-supported scientists found that LCA can bind to and activate a protein called the vitamin D receptor, so-named because it is a steroid receptor that binds the steroid hormone vitamin D. Once activated, the vitamin D receptor turns on genes. Intriguingly, one gene thought to be controlled by the vitamin D receptor encodes an enzyme that detoxifies LCA. When the scientists gave LCA to mice, the gene for this enzyme was turned on in the liver and intestines of the animals. Vitamin D activated its receptor and then this enzyme in a similar fashion. This research revealed a previously unknown mechanism for eliminating LCA in which the vitamin D receptor acts as a sensor for LCA. By binding to the vitamin D receptor, both LCA and vitamin D may boost production of an enzyme that detoxifies LCA in the digestive tract. These findings may help explain the protective effects of vitamin D against colon cancer.

These studies show that two different natural compounds that counteract some of the effects of high fat and cholesterol both interact with proteins that turn on genes which influence either bile acid production or detoxification. With these insights, opportunities may emerge for developing new therapeutic strategies to target the molecular pathways through which vitamin D and guggulsterone exert their effects.

Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, and Mangelsdorf DJ: Vitamin D receptor as an intestinal bile acid sensor. *Science* 296: 1313-16, 2002

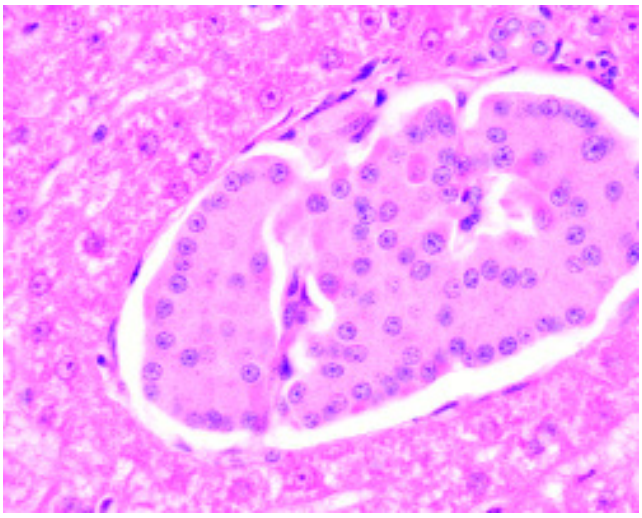
Urizar NL, Liverman AB, Dodds DT, Silva FV, Ordentlich P, Yan Y, Gonzalez FJ, Heyman RA, Mangelsdorf DJ, and Moore DD: A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science* 296: 1703-6, 2002.

DEVELOPING STRATEGIES TO OVERCOME IMMUNE REJECTION OF TRANSPLANTED CELLS AND ORGANS

Critical to the success of any transplantation procedure is survival of the transplant in the recipient. However, the body's immune system is programmed to attack foreign material that enters the body—whether this material is infectious microbes, such as bacteria and viruses, or potentially life-saving organs. This problem has long plagued transplant patients, because agents used to suppress the immune system to prevent transplant rejection can also leave the patient more vulnerable to infection and other types of complications. Recently, scientists have obtained promising results in investigations of new drugs and drug combinations designed to modulate the immune system.

Scientists have developed a primate model of islet transplantation, a potential therapy for type 1 diabetes, based on an immunosuppressive strategy first developed in Edmonton, Canada, for human islet transplantation. An animal model that is quite similar to humans will facilitate further evaluation and refinement of this still-experimental procedure for restoring insulin-producing capacity to patients whose own insulin-producing cells have been destroyed by an aberrant immune system.

The scientists first induced diabetes in macaque monkeys, and then infused the monkeys with islets from other macaques. They also administered three immununosuppressive agents used in the human procedure: daclizumab, FK506 (tacrolimus), and



This is a cross-section of a pancreatic islet (rimmed in white) that was transplanted into a diabetic macaque monkey. The nucleus of each cell in the islet is stained dark purple. Researchers are working to improve methods for the transplantation of insulin-producing islets as one approach to treating or curing type 1 diabetes.

Photo: Dr. Boaz Hirshberg. Copyright © 2001 American Diabetes Association. From *Diabetes,* Vol 50, 2001; 2231-2236. Reprinted with permission from the American Diabetes Association.

rapamycin. In monkeys that maintained effective levels of these agents in their blood, relief from the symptoms of diabetes was achieved. The immunosuppressive agents had subdued their immune systems into accepting islets from another animal, reproducing the effects seen in humans. Already, the scientists have gained important information from this primate model: they confirmed that the site of injection currently used to transplant islets into a recipient, the portal vein of the liver, is superior to another site.

Scientists are also investigating a new type of immunosuppressive agent to induce immune tolerance. This agent is a monoclonal antibody that binds to an important immune-cell protein called CD154. In recent years, the scientists demonstrated that anti-CD154 monoclonal antibodies are extraordinarily efficacious in preventing rejection of kidney transplants in monkeys. This past year, they

put anti-CD154 antibodies to an even more rigorous test—skin transplantation. The scientists first selected pairs of donor and recipient rhesus monkeys that differed genetically in ways most likely to provoke immune rejection, to mimic a transplant between two entirely unrelated people. They then transplanted skin grafts onto the animals, and treated them with anti-CD154 antibodies. The anti-CD154 antibodies greatly enhanced the survival of the skin grafts in the transplant recipients.

The success of many transplantation procedures has been predicated on research into immune intervention to prevent transplant rejection. Immunosuppressive agents play an additional role in "autoimmune" diseases such as type 1 diabetes. Because type 1 diabetes results from destruction of pancreatic islets by an aberrant immune system, immunosuppressive agents are needed not only to reduce transplant rejection, but also to help avert a recurrence of the immune attack that caused the disease in the first place. The continued exploration of immunosuppressive agents in animals will likely translate into improved human health, not only through advances in immune modulation, but also through the development of animal models useful for optimizing other aspects of transplant procedures.

Elster EA, Xu H, Tadaki DK, Montgomery S, Burkly LC, Berning JD, Baumgartner RE, Cruzata F, Marx R, Harlan DM, and Kirk AD: Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primates. *Transplantation* 72: 1473-8, 2001.

Harlan DM: Islet cell allotransplantation as a model system for a bioengineering approach to reparative medicine: Immunological concerns. *Ann N Y Acad Sci* 961: 331-4, 2002.

Hirshberg B, Montgomery S, Wysoki MG, Xu H, Tadaki D, Lee J, Hines K, Gaglia J, Patterson N, Leconte J, Hale D, Chang R, Kirk AD, and Harlan DM: Pancreatic islet transplantation using the nonhuman primate (rhesus) model predicts that the portal vein is superior to the celiac artery as the islet infusion site. *Diabetes* 51: 2135-40, 2002.

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY FOR HIV INFECTION—BENEFITS AND DRAWBACKS

Current therapy for infection with the human immunodeficiency virus (HIV) usually involves a multi-drug regimen known as highly active antiretroviral therapy (HAART). This therapy is very effective at inhibiting proliferation of HIV, thus preserving patients' immune systems and general health and improving survival. However, prolonged treatment with HAART is associated with a potentially serious metabolic syndrome that may result in the redistribution of body fat (lipodystrophy), lipid abnormalities, and insulin resistance or diabetes. These complications of HAART, and in particular the distressing physical changes induced by lipodystrophy (see also the "Story of Discovery: Leptin—A Potential Treatment for Lipodystrophy"), can cause some patients to stop this life-preserving treatment. The NIDDK is supporting studies that address these metabolic complications of HAART, in order to neutralize the drawbacks and maintain the beneficial effects of this therapy for patients.

Human Growth Hormone and Insulin Resistance: Researchers have shown that treating HIV-positive individuals who have central fat accumulation with human growth hormone (hGH) reduces total body and visceral fat and increases lean tissue mass. However, a decrease in insulin sensitivity was noted in these individuals after 1 month of hGH therapy, detracting from its benefits. In a recent study, NIDDK-supported researchers examined the contribution of the liver to changes in insulin sensitivity, as well as changes in fat metabolism in these same individuals prior to and after 1 and 6 months of hGH therapy. In these patients, hGH treatment improved the overall lipid profile, resulting in a decrease in triglycerides and total cholesterol and an increase in HDL ("good") cholesterol concentration. However, hGH treatment was also associated with insulin resistance in the liver and other tissues.

This study clarifies the potential risks and benefits of hGH therapy of the metabolic syndrome associated with HIV and its therapy, demonstrating that improvement in some parameters may be accompanied by worsening of others. By elucidating the trade-offs and complexities involved, this work has implications for the design of therapeutic strategies to mitigate the metabolic effects of HAART.

The HAART of Insulin Resistance: HAART is a cocktail of drugs that inhibit essential enzymes the HIV virus uses to infect and replicate within cells. One of the viral enzymes targeted by HAART is called a protease. Previous studies have suggested that a component of many HAART regimens, the protease inhibitor (PI) indinavir, can cause insulin resistance in the absence of other physiologic changes and even in the absence of HIV infection.

To investigate this hypothesis, NIDDK-supported researchers recently examined changes in responsiveness to insulin in six healthy, HIV-negative men in the presence and absence of the PI indinavir. The six men participated in a double-blind, crossover study assessing the effects of indinavir or placebo on insulin sensitivity. The dose of indinavir used in the experiment was similar to that used in a typical HAART regimen. Changes in insulin sensitivity were measured using a procedure in which study participants receive a steady, relatively high dose of insulin—used to generate a hyperinsulinemic state and promote continuous uptake of glucose by tissues from the blood—and a variable dose of dextrose, a form of glucose. Blood sugar levels are monitored throughout the experiment and the rate of dextrose infusion is adjusted to maintain constant blood sugar levels (euglycemia). The more dextrose that must be infused to maintain euglycemia—i.e., to replace glucose that has been taken up by tissues—the greater the individual's responsiveness to insulin.

The researchers found that the volunteers who received the indinavir required a significantly lower rate of dextrose infusion to maintain euglycemia compared to those who received placebo. Most of the difference in glucose metabolism in the indinavir group was due to a drop in the amount of "non-oxidative glucose disposal"—precisely the kind of glucose metabolism that typically occurs in skeletal muscle, a major target for insulin action.

Previous studies have indicated that indinavir and other PIs can inhibit the uptake of glucose by cultured fat cells through inhibition of the glucose transporter GLUT4. Indinavir has also been shown to inhibit glucose uptake by isolated rat muscle in culture. The current study indicates that the PI indinavir can rapidly and significantly impair glucose metabolism in humans, at least in part through inhibition of glucose disposal by skeletal muscle. The evidence suggests a direct effect on some aspect of the insulin-signaling pathway because the change in insulin responsiveness is seen so quickly following administration of a single dose of indinavir.

These studies offer important insights into the understanding of the physiological causes of HAART-associated metabolic complications and may lead to the refinement of HIV therapeutic approaches. To encourage more research in the fundamental biochemical or pathogenic mechanisms of the metabolic complications associated with HIV-disease and antiretroviral therapy, the NIDDK is collaborating with other NIH Institutes on the initiative, "Complications of Antiretroviral Therapy." It is anticipated that research studies supported through this initiative may lead to improved medical management of metabolic complications with existing agents and potentially may lead to the design of agents or treatment strategies less likely to produce such complications.

Noor MA, Seneviratne T, Aweeka FT, Lo JC, Schwarz JM, Mulligan K, Schambelan M and Grunfeld C: Indinavir acutely inhibits insulin-stimulated glucose disposal in humans: A randomized, placebo-controlled study. *AIDS* 16: F1-8, 2002.

Schwarz JM, Mulligan K, Lee J, Lo JC, Wen M, Noor MA, Grunfeld C, and Schambelan M: Effects of recombinant human growth hormone on hepatic lipid and carbohydrate metabolism in HIV-infected patients with fat accumulation. *J Clin Endocrinol Metab* 87: 942-5, 2002.

"CONSIDERING THAT EXTRA HELPING?" GUT HORMONES THAT INFLUENCE WEIGHT REGULATION

Long-term control of body weight is achieved through balancing food intake and physical activity. The "appetite control center" in the brain monitors an array of chemical signals generated by fat, muscle, and other tissues in order to assess energy needs and stores and to modify appetite and physical activity accordingly—thereby ensuring energy balance (see also "Digestive Diseases and Nutrition" chapter). The gut plays a key role in the control of body weight not only by absorbing nutrients, but also by providing both hunger and satiety signals. Many of these signaling molecules are just now being identified.

Ghrelin is a hormone secreted by the stomach and upper intestine that stimulates appetite. Bloodstream levels of ghrelin cycle throughout the day, peaking just before a meal and declining to a pre-set baseline level soon after. Recent studies in both animals and normal human volunteers reinforce a role for this hormone as a signal to eat.

In one study, NIDDK-supported researchers found that, in response to weight loss of 17 percent, dieters' overall ghrelin levels increased by an average 24 percent—a compensatory response typically observed with signaling molecules involved in maintaining energy balance. This may explain why weight loss is so difficult for dieters. Complementing this finding, these investigators discovered that ghrelin secretion is nearly non-existent in persons who have had Roux-en-Y gastric bypass (RGB) surgery, a treatment for severe obesity in which the top of the stomach is surgically connected to the middle of the intestine. This observation is consistent with the decrease in appetite experienced by the majority of RGB surgery patients, and may explain their success in maintaining long-term weight loss.

In related work, the group examined ghrelin levels in patients with Prader-Willi syndrome (PWS), a genetic disorder causing the most common form of human syndromic obesity. Persons with diet-induced obesity have what appears to be a compensatory drop in baseline ghrelin levels. In contrast, the researchers found that PWS patients have significantly elevated baseline levels of ghrelin—suggesting that derailed regulation of ghrelin secretion may contribute to weight gain in this disorder.

In contrast to ghrelin, the gut hormone PYY_{3-36} appears to inhibit appetite. This hormone is secreted by the intestine in response to a meal. NIDDK-supported researchers recently found that injecting PYY_{3-36} to achieve levels similar to those after a meal had long-term effects on energy balance: it decreased total food consumption and reduced weight gain in rats, and inhibited appetite and reduced food consumption in humans. The latter was strikingly demonstrated by the observation that individuals who were injected with PYY_{3-36} 2 hours before a free choice buffet meal consumed approximately one-third fewer calories than persons not given the hormone, an effect which lasted several hours.

The research team further characterized the activity of the hormone in the brain pathways regulating appetite. They found that PYY_{3-36} indirectly stimulates specific cells (POMC neurons) in the pathway that leads to appetite inhibition. Furthermore, mice lacking a putative brain cell receptor for PYY_{3-36}, known as Y2R, no longer showed appetite inhibition when injected with PYY_{3-36}. These results suggest a possible molecular mechanism for the observed activity of PYY_{3-36}.

An estimated 64 percent of American adults are overweight or obese, a strong risk factor for heart disease and type 2 diabetes. These advances demonstrate a strong relationship between levels of two gut hormones, ghrelin and PYY_{3-36}, and appetite. Because appetite control is one of the greatest challenges for dieters, understanding the activities of ghrelin and PYY_{3-36} in the body may prove to be an important key for developing interventions to control weight or to achieve and sustain weight loss.

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, and Bloom SR: Gut hormone PYY_{3-36} physiologically inhibits food intake. *Nature* 418: 650-4, 2002.

Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A, and Weigle DS: Elevated plasma ghrelin levels in Prader Willi syndrome. *Nat Med* 8: 643-4, 2002.

Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, and Purnell JQ: Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 346: 1623-30, 2002.

IDENTIFICATION OF A MEDIATOR OF LEPTIN ACTION IN MUSCLE

Another hormone involved in energy storage, and hence weight regulation, is leptin. Leptin, secreted by fat cells, plays a pivotal role in the regulation of food intake and energy metabolism. Leptin was first shown to act on a region of the brain known as the hypothalamus to reduce food intake—the first fat hormone shown to have such an important role in regulating energy balance. Subsequently, other effects on peripheral metabolic tissues were identified. Leptin was found to promote fat metabolism and the uptake of glucose from the blood. However, the signaling pathways through which leptin exerts these effects on cellular metabolism are largely unknown.

Fatty acids within a cell may be either oxidized—used as fuel—or stored. In the muscle, activation of the enzyme acetyl coenzyme A carboxylase (ACC) tips the energy balance toward fat storage. The activity of ACC is in turn regulated by 5'-AMP-activated protein kinase (AMPK), an enzyme that, when activated, inhibits ACC. Activation of AMPK therefore tips the energy balance away from fat storage and toward fat burning. In a significant recent research advance, leptin has been identified as an activator of AMPK in muscle, and may thus promote fat burning through AMPK's antagonism of ACC activity.

After infusing leptin into the brains of intact mice, NIDDK-supported scientists noted an increase in AMPK activity in muscle, peaking 1 hour after the injection and persisting for up to 6 hours. When leptin was infused intravenously into mice, muscle AMPK activity also increased, but the response was different: AMPK activity doubled after 15 minutes, returned to baseline after 1 hour, and slowly doubled again over the next 5 hours. Scientists then cut the connections between the nervous system and the leg muscles of mice. They found that AMPK activity in leg muscles still rose quickly following intravenous leptin infusion but did not rise following injection of the hormone into the brain. Taken together, these observations strongly suggest that leptin regulates muscle metabolism through at least two mechanisms of action: a rapid, direct effect on the muscle itself and a slower, indirect effect mediated through the nervous system.

Leptin's role was first described as a signal to limit food intake and it was considered a promising possible therapy for obesity. Unfortunately, in clinical trials, leptin did not cause significant weight loss except in rare genetic forms of obesity, and it has since been found that many obese people are functionally leptin resistant. The identification of AMPK as a downstream player in the leptin signaling pathway might open the door for the development of novel therapies aimed at this molecule for the treatment of obesity (see also "Digestive Diseases and Nutrition" chapter). Ongoing research will determine whether AMPK-targeted therapies can bypass the problem of leptin resistance and might therefore be more effective in the treatment of obesity than administering leptin itself.

Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Müller C, Carling D and Kahn BB: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415: 339-43, 2002.

OF MICE AND MUSCLES: INCREASING STAMINA THROUGH GENE REGULATION

Adult skeletal muscles are made up of two main types of fibers that convey different properties to the various muscles in the body. Type II ("fast-twitch") muscle fibers use the sugar glucose as a main energy source and fatigue rapidly, while type I ("slow-twitch") muscle fibers contain many high-energy-producing cellular components known as mitochondria and are less susceptible to fatigue. As a source of energy, type I muscle fibers use oxidative metabolism, a chemical process in which oxygen is used to break down sugars, yielding more energy than the process used by type II fibers. The amount of each fiber type in each muscle appears to be regulated. For example, exercise, such as endurance training, can increase the proportion of type I fibers found in leg muscles.

To learn how muscle fiber type is determined, scientists induced changes in mouse muscle fiber types—not with athletic training, but with transgenic technology. Knowing from previous research that the protein PGC-1alpha is important in the production of mitochondria, the scientists engineered transgenic mice to boost levels of PGC-1alpha in certain leg muscles that normally contain a large portion of type II fibers. When scientists examined the muscles in the transgenic mice, they discovered an increased level of expression (turning on) of genes that encode mitochondrial proteins, as well as other distinctive type I fiber proteins. The muscles of the transgenic mice also showed improved endurance. The scientists then went on to use isolated muscle cells to identify some of PGC-1alpha's partners in activating gene expression in muscle. These studies show that PGC-1alpha is a major regulator of type I muscle fiber determination. Additionally, because muscles take up and use glucose, and because impaired glucose uptake in response to insulin is associated with type 2 diabetes and obesity, the scientists speculated that pharmacological manipulation of muscle fiber-type determination may have implications for these medical conditions.

Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, and Spiegelman BM: Transcriptional co-activator PGC-1alpha drives the formation of slow-twitch muscle fibres. *Nature* 418: 797-801, 2002.

ONGOING AND NEWLY LAUNCHED NIDDK EFFORTS IN DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES

Capitalizing on and responding to recent advances and emerging opportunities, the NIDDK continues to foster cutting-edge research in diabetes, endocrinology, and metabolic diseases—from the recognition and support for innovative ideas in investigator-initiated research, to the Institute-led establishment of consortia focused on important fundamental and clinical research issues.

The NIDDK will provide support for the use and development of new proteomics technologies for studying diabetes and other endocrine and metabolic diseases. While gene microarray technology (GMT) and other advanced genomics methods continue to give researchers critical "snapshots" of gene expression in cells under different conditions, proteomics technologies are delivering a wealth of information about the expression, modification, interactions, and destruction of the primary products of gene expression—proteins.

To accelerate fundamental research in diabetes, the NIDDK recently established the Beta Cell Biology Consortium, a collaborative effort to facilitate interdisciplinary approaches that will advance understanding of pancreatic islet development and function. The hope is that an increased basic understanding of the islets and the beta cells within them will accelerate clinical efforts to replace, preserve, or even "re-program" them—benefiting both type 1 and type 2 diabetes patients. The NIDDK also plans to enhance research on fat cells and fat tissue, important for combating obesity—a pathological condition on its own and a major risk factor for type 2 diabetes, as well as for heart

disease and certain cancers. In collaboration with the National Institute of Aging, the NIDDK recently issued a research solication, "The Life Cycle of the Adipocyte," to encourage basic research studies on fat cell (adipocyte) biology.

As mentioned previously, the Type 1 Diabetes TrialNet is completing a trial begun by the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1) to determine whether oral insulin administration can delay or prevent the onset of type 1 diabetes in individuals at risk for the disease. TrialNet will also begin studies aimed at preserving beta cell function in patients with new-onset type 1 diabetes. Another NIDDK-supported trial for therapy of type 1 diabetes is testing a regimen of steroid-free immunosuppression to prevent islet cell rejection following islet transplantation. A new long-term clinical effort, TEDDY (Triggers and Environmental Determinants in Diabetes of the Young), is a consortium set up to identify infectious agents, dietary factors, or other environmental factors that trigger the development of type 1 diabetes in genetically susceptible individuals. It is hoped that the TEDDY study will generate new targets for therapy.

The NIDDK-led Diabetes Prevention Program (DPP) clinical trial, completed in 2001, demonstrated that type 2 diabetes could be delayed or prevented with either lifestyle modification or metformin in adults at high risk, including minorities who suffer disproportionately from the disease. The NIDDK has now initiated a long-term follow-up study to address the durability of the DPP interventions in preventing or delaying diabetes and to determine whether the interventions reduce cardiovascular disease and atherosclerosis. In conjunction with the National Heart, Lung, and Blood Institute, the NIDDK is also supporting the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial. This randomized, multi-center trial will study three key approaches to preventing major cardiovascular events in individuals with type 2 diabetes, targeting control of blood glucose, blood pressure, and lipid levels.

To address the alarming rise in type 2 diabetes in children—which parallels the increase in overweight and obesity in this population—the NIDDK will begin multi-center clinical trials on prevention and treatment strategies. A pilot study is planned to test leptin as a treatment for children with severe insulin resistance syndrome, because of leptin's positive effects on glucose uptake.

To foster collaborations between clinical and basic scientists with the goal of translating fundamental research advances into new therapies, the NIDDK reissued a "bench-to-bedside" research initiative on type 1 diabetes and its complications. The NIDDK also recently established training and career development programs in diabetes research for pediatric endocrinologists. Another effort will strive to develop more effective ways to translate into improved health care practices the results of large clinical trials such as the DPP, for type 2 diabetes, and the Diabetes Control and Complications Trial (DCCT), for type 1 diabetes. These translation efforts will be complemented by the ongoing educational activities of the National Diabetes Education Program, which include disseminating the positive results of the DPP trial to the public at large.

Finally, in an important endocrinology research area, the NIDDK will support a new effort to pursue increased knowledge of androgen (male sex hormone) receptor signaling in the prostate, in order to better understand prostate growth and cancer—complementing its ongoing efforts on benign prostate disease (see "Kidney, Urologic, and Hematologic Diseases" chapter).

PATIENT PROFILE

Mollie Singer

Living with Type 1 Diabetes

It happened the day before then 10-year-old Mollie Singer was to testify before Congress on behalf of the 1999 Children's Congress of the Juvenile Diabetes Research Foundation International. Mollie was a guest in the Senate gallery absorbing the legislative process. She was seated next to her mother and a senator's wife when her mother happened to clasp Mollie's hand and felt it cold and clammy to the touch. "I took one look at Mollie, tested her right there in the gallery and realized she was going into a low blood sugar emergency," says Mrs. Singer. "I literally had to pick her up, rush her out into the hallway and immediately give her glucose to raise her blood sugar." All this, while the sympathetic senator's wife looked on with great concern. Had Mrs. Singer not acted as she did, Mollie, who was already beginning to feel groggy and disoriented due to the sudden drop in her blood sugar, ran the very real risk of passing out, going into convulsions, and slipping into a diabetic coma. The very next day, Mollie testified before Congress, asking representatives to "promise to remember me" when they provide resources for biomedical research.

This is the story of a very courageous little girl and her equally courageous family who battle with type 1 diabetes every minute of their lives. The family lives in constant fear, walking a tightrope between the potentially deadly complications that can steal vision, limbs, and years of productive life from their loved one on the one hand, and the immediate danger of swings in blood sugar levels on the other. And they are doing everything in their power to help each other, and the 17 million other Americans with type 1 and type 2 diabetes, live better, fuller, and more hopeful lives.

Since Mollie Singer was diagnosed with type 1 diabetes when she was four-and-a-half years old, she has been struggling to manage the disease every day of her life. Mollie at right, Jackie at left.

"My DREAM is that the doctors find the cure for diabetes," Mollie Singer wrote at age 11, nearly 7 years after she was diagnosed with type 1 diabetes. "When that happens... I'll be so happy, I'll cry!!... NO MORE SHOTS!... I would like to know what it feels like not to have this horrible disease and just be a regular kid."

"Diabetes is something I have to live with," says Mollie, now 13 years old. "And," she adds in her upbeat, giggly adolescent voice that belies the strength and dedication behind it, "I'm going to do everything I need to do to stay alive and live a good life until they find a cure for me and other children with this disease." The fact is, she and her fraternal twin sister, Jackie, are true profiles in courage, commitment, and love when it comes to fighting the good fight against diabetes.

MOLLIE SINGER

"Diabetic (Guardian) Angels"
"I hate that my sister has this disease," says Jackie, who does not have diabetes. Nor is there a history of the disease in the adopted twins' biological family. "It's hard to watch Mollie go through all the pain. I don't sleep nights because I'm afraid that something will happen to her." Jackie's fears are not unfounded. Over the past several years, Jackie has had to rouse her parents in the middle of the night on several occasions when she realized that Mollie's blood sugar had dropped to dangerously low levels. While mother and father tended to Mollie's urgent health needs, Jackie called 911. When paramedics arrived at the door, Jackie described to them what her twin sister was going through. "Jackie is my guardian angel," says Mollie, with deep affection. "She's always watching over me. She's totally cool."

S-o-o-o cool, that when the twins were in fourth grade they started a club they called Mollie's and Jackie's Diabetic Angels to educate other kids about diabetes, and to get them to become guardian angels for Mollie and others with the disease. In class, for example, if the teacher forgot, the kids would yell, "It's time for Mollie to test her blood sugar." The 40 or so classmates who joined the club also wrote to their congressional representatives in support of research dollars to find a cure for diabetes. Never ones to miss out on an opportunity to educate others about diabetes, Mollie and Jackie wrote up a plan and mission statement for their club and used the Internet to promote it. Today, according to Mrs. Singer, who gave up her profession as a film production consultant after Mollie was diagnosed with type 1 diabetes in 1993, there are more than 20 Diabetic Angels clubs around the country, as well as in Australia and Israel.

"My twin sister Jackie wonders how many more birthdays we will celebrate, before someone finds the cure [for diabetes]," says Mollie. "It makes me so sad that Jackie can't be a regular kid either, because she is always worrying about me."

The rules and mission statement Mollie and Jackie established for their club speak volumes about their love and dedication to one another, and their commitment to seeking a cure for diabetes for others. The original club rules read as follows:

- Know what it means when Mollie says her blood sugar is high or low and also know what to do to help her.
- You have to know how to test her in case she's having trouble testing herself and I'm (Jackie) not around.
- You have to agree to write a short letter to our representatives when it's necessary and ask them to please give more money for diabetes research.
- Help raise funds for research by walking...in the Juvenile Diabetes Research Foundation's "Walk to Cure Diabetes" if your parents say it's O.K.
- And the last rule is, represent the Diabetic Angels with honor. This means accepting the differences in all people and be a kind and understanding person.

Starting the club was just the first salvo in the twins' never-ending war against diabetes. To raise awareness about the disease and its deadly complications, these two adolescent dynamos have met with President George W. Bush and appeared on TV's Good Morning America, and they routinely take part in the annual Juvenile Diabetes Research Foundation's "Walk to Cure Diabetes," appear in documentaries, do interviews, stay current on research and political issues related to diabetes—writing letters to Members of Congress whenever they feel it's necessary—and more. "At night, Jackie and I pray for everyone who is sick," says Mollie, "and we ask God to help the doctors find the cure for diabetes and other terrible diseases."

MOLLIE SINGER

Mollie's and Jackie's Diabetic Angel's Mission Statement
The goal of a "Diabetic Angel" is to support his or her diabetic friend...be prepared to help in an emergency...bring about awareness by educating classmates, friends, and parents...and help raise funds for diabetes research until diabetes is cured!

Living with the Disease
Mollie's and Jackie's activism is a direct result of the lack of understanding and insensitivity Mollie encountered shortly after she was diagnosed with type 1 diabetes—sometimes referred to as juvenile diabetes—at age four. When the twins were five, Mollie was in the hospital for open-heart surgery, which was unrelated to her diabetes. "I had a real bad time," Mollie recalls. "No one knew how to handle a child with diabetes, so I got the wrong amount of insulin and the wrong food." In school, she's been embarrassed when her high blood sugar has made her vision blurry, making it hard for her to read, and people have told her point-blank that "diabetic kids are a hassle."

If the misunderstandings and insensitivities aren't enough, consider the fact that, from the time she was diagnosed until the day she received an insulin pump in January 2000, Mollie had been injected with 12,889 shots of insulin and had her little fingers poked more than 25,000 times in order to take her blood sugar readings. "Everything I do is planned around my diabetes," says Mollie, including eating, sleeping, playing, and even homework. "If things are not planned exactly, my blood sugar levels can go out of control." Just ask her parents. "In the past, when we would go to restaurants," says Mollie's father, Dr. Singer, an anesthesiologist, "I'd always worry about how long it would take for us to get our food. Sometimes I'd see Mollie crashing right in front of me." Mrs. Singer quickly adds that ladies' rooms, airplanes and cars are horrible places to give insulin shots.

For years after she was first diagnosed, the only item on Mollie's Christmas gift wish list was a cure for diabetes. "Finding a cure for diabetes is all I think about every hour of every day," says Mollie. "I try to be brave but sometimes I get very sad and cry myself to sleep."

All that has been mitigated somewhat since Mollie began using an insulin pump nearly 3 years ago. Proper use of the device takes a relatively high degree of awareness and responsibility, including the ability to count carbohydrate intake. However, the pump has changed Mollie's life. She no longer needs to take insulin shots four-to-six times a day, nor carry around the syringes, alcohol pads and other supplies necessary for injections. She's also able to eat foods she wasn't able to eat before. In short, although she still needs to test her blood sugar levels regularly, the pump has introduced lots of freedom into Mollie's and her family's lives. "My pump looks like a beeper, it's so cool," says Mollie. To make it even cooler, Jackie, of course, adorns Mollie's pump cases using silver pens, colorful materials and little patches. "The pump makes having the disease a little less painful for Mollie," says the ever-loyal Jackie.

NIDDK-supported clinical research studies have demonstrated that controlling blood sugar levels for even a few years can significantly mitigate the complications of diabetes—including nerve, eye, and kidney disease—later in life. The recent development of the insulin pump, also with NIDDK support, has made it easier for insulin-dependent diabetics to manage their blood sugar levels throughout the day, thus reducing the risk for developing complications.

Always in the vanguard, Mollie and Jackie are eagerly awaiting the day Mollie can use an implantable insulin pump, which is still in clinical trials. They are also excited about the many scientific advances being made in diabetes research, including promising studies of islet transplantation, better ways to monitor blood sugar levels at home, medicines that can prevent or delay complications in people with diabetes, and more.

"Every night, night after night, I have the same routine," says Mollie. "I pray for the cure and dream about what that day will be like. The cure is all I dream about, because my future depends on whether or not my dream comes true."

STORY OF DISCOVERY

Leptin—A Potential Treatment for Lipodystrophy

When researchers discovered the mouse obesity gene and its protein product, leptin, they unleashed a tidal wave of new research advances in fat biology and metabolism. The discovery that leptin is secreted by fat cells and is released in proportion to the amount of fat drastically altered the former view of normal fat tissue as a passive "fat storehouse." Research fueled by this 1994 discovery has also led to the identification of a number of other substances that, like leptin, are secreted by fat cells and influence appetite and metabolism. Leptin is secreted into the bloodstream where it travels to the brain and signals the body to reduce food intake. Leptin may also affect one's food preferences and lessen one's craving for sweets. Leptin additionally affects the liver, muscle, and pancreas—organs that influence the body's ability to use fats and sugar. It can suppress the activity of an enzyme necessary for fat production and improve the sensitivity of muscle and other tissues to insulin, a hormone that regulates the body's storage and utilization of glucose, a key energy source.

Animals genetically deficient in leptin were found to be extremely obese. Because the animals lost weight when given leptin, researchers postulated that leptin treatment might also be useful for human obesity. There are, in fact, very rare instances of complete deficiency of leptin in humans, resulting in morbid obesity from infancy. Leptin treatment in these individuals caused substantial weight loss, providing hope for improved quality of life and longevity.

Unfortunately, in clinical studies, leptin has not proven to be the panacea for the treatment of obesity in the vast majority of cases. With rare exceptions, obesity generally results from a complex interaction between our genes and our environment and lifestyle—particularly eating too much and exercising too little. Obese individuals, in fact, usually have very high levels of leptin, probably reflecting the many fat cells secreting it. The failure of all this leptin to decrease body weight suggests that the more common forms of obesity are associated with a resistance to leptin's actions.

Although leptin has not been a successful treatment for most cases of obesity, it has shown therapeutic promise for other disorders. A particularly fascinating example is lipodystrophy. This is actually a group of disorders with disparate origins but with a common set of characteristics. Individuals with lipodystrophy lack fatty tissue in the face, neck or extremities; they sometimes have central obesity and sometimes lack fat tissue altogether. These patients exhibit resistance to the effects of insulin and are at high risk of developing diabetes. They may also have a range of lipid abnormalities. Treatment of lipodystrophy has included insulin, oral hypoglycemic (blood sugar lowering) agents, and lipid-lowering drugs. In spite of treatment, patients continue to have severely high levels of triglycerides, leading to recurrent attacks of acute inflammation of the pancreas; severe problems controlling blood sugar levels, posing risks of diabetic eye and kidney disease; and fat accumulation in the liver, which can result in cirrhosis and liver failure. Because many lipodystrophy patients have low leptin levels, and because recent studies have demonstrated beneficial effects of leptin on insulin sensitivity and fat metabolism in a number of tissues, researchers investigated whether leptin treatments could ameliorate conditions associated with lipodystrophy.

STORY OF DISCOVERY

Two recent publications reported exciting preliminary results of leptin treatment in small clinical studies of individuals with lipodystrophy. In one study, scientists found that leptin therapy markedly improved insulin sensitivity, lowered lipid levels, and decreased fat in the liver in individuals with severe lipodystrophy who also suffered from poorly controlled type 2 diabetes. After leptin treatment, the patients were also able to discontinue their diabetes medications. In addition, the patients had decreased appetite. In concomitant studies of lipodystrophy in animals, researchers found that leptin deficiency could explain most if not all of the confusing metabolic disturbances seen in this disorder.

In another study, researchers tested the effect of leptin in female patients with different forms of lipodystrophy, most of whom also had type 2 diabetes. During the study, most of the patients experienced significant improvements in their blood glucose levels, which in turn lowered their risk of developing diabetic eye and kidney complications. The leptin therapy also reduced their triglyceride levels. Liver size also decreased, indicating a loss of stored fat. Patients were able to reduce or stop using drugs to control their diabetes, and they reported eating less following treatment. Because of the dramatic improvement in their quality of life, the individuals in this study are continuing to receive leptin therapy.

Lipodystrophy can either be inherited or acquired. Researchers recently identified the genes responsible for two forms of inherited lipodystrophy; these findings may provide new therapeutic targets for lipodystrophy and other metabolic disorders. Lipodystrophy is often acquired by people infected with the human immunodeficiency virus (HIV) who are undergoing treatment with highly active antiretroviral therapy (HAART). Although HAART has dramatically improved the survival of people with HIV, it is associated with a variety of metabolic complications, including elevated fat levels in the blood, insulin resistance, osteoporosis (bone loss), and lipodystrophy. The earlier success with leptin in treating lipodystrophy provides hope that it may be effective in HIV-associated lipodystrophy as well.

While lipodystrophy is characterized by loss of fatty tissue in certain areas of the body, tissues such as liver and muscle exhibit significant abnormal accumulation of fat which impairs metabolic activity. Another condition marked by inappropriate accumulation of fat in the liver is non-alcoholic steatohepatitis (NASH), a disease most common in overweight adults over 40 years of age. Individuals with NASH have insulin resistance, elevated levels of fats in their blood and a high risk of developing diabetes. Like obesity (but unlike lipodystrophy), NASH is correlated with high leptin levels. Thus, leptin resistance may play a role in the development of this disease.

The discovery of leptin has led to a cascade of exciting and unexpected findings with broad implications for the successful treatment of disease. While the initial excitement was tempered by the lack of success in countering obesity, leptin is now proving efficacious for treating less common disorders such as severe lipodystrophy. The promise that accompanied the discovery of leptin may yet be fulfilled, as future studies that do lead to effective tools to combat obesity will likely trace their origins to this remarkable discovery.

Diabetes Education at NIDDK: The National Diabetes Education Program

Although making new discoveries about diseases and how to prevent them is one of NIH's most important missions, these discoveries can't benefit Americans if they're not put into practice to improve health. Thus, education programs directed at health-care providers and the public are also an important part of NIH's mission.

Diabetes is a major nationwide epidemic in the U.S.—approximately 17 million Americans suffer from it—and projections indicate that if current trends persist, its incidence will increase 165 percent by the year 2050. The majority of Americans suffering from diabetes have type 2 diabetes, also known as non-insulin-dependent diabetes. The National Diabetes Education Program (NDEP) is a collaborative initiative of the NIDDK and the CDC that uses over 200 public and private partnerships to promote application of research findings that have demonstrated value in the prevention of type 2 diabetes and diabetic complications.

A key feature of the NDEP is the participation of older adults and individuals who represent communities such as African Americans, Hispanics/ Latinos, American Indians/Alaska Natives, and Asian and Pacific Islanders, who are disproportionately affected by type 2 diabetes. At present, the NIDDK is working to convey the important, science-based diabetes prevention findings of the Diabetes Prevention Program (DPP) to help reverse the rising tide of diabetes in this country.

Sponsored by the NIDDK, the DPP showed that people with pre-diabetes—those whose blood glucose levels are higher than normal but not yet diabetic—can delay and possibly prevent type 2 diabetes by losing 5-to-7 percent of their body weight through moderate improvements in diet and exercise. The DPP found that modest weight loss and regular physical activity, such as brisk walking for 30 minutes a day, five times per week, could cut the risk of developing type 2 diabetes by more than half in people with pre-diabetes. These lifestyle changes worked for people of every ethnic or racial group who participated in the study, and they were especially successful for people over age 65. The NDEP's challenge now is to translate the prevention message of the DPP to at-risk persons throughout the country.

A new national diabetes prevention campaign, launched on November 20, 2002, by HHS Secretary Tommy Thompson, will be coordinated by the NDEP. The program, entitled "Small Steps, Big Rewards," represents the first major NDEP effort to translate the DPP results on a national level, and its message is targeted at the 16 million Americans most at risk—those who have pre-diabetes.

The program emphasizes the practical application of the DPP findings and includes lifestyle-change tools for those at risk, patient education materials for healthcare providers, web-based resources for both healthcare providers and consumers, and TV, radio and print public service announcements. The NDEP will be tapping its partners at local, state and national levels for help in disseminating the new program's message, and will also recruit businesses and consumer-based programs as partners in this effort.

While working to increase awareness about diabetes and effective means for prevention, the NDEP is continuing a core campaign, "Be Smart About Your Heart: Control the ABCs of Diabetes." This campaign is designed to make people with diabetes aware of their high risk for heart disease and stroke—the leading causes of death in these patients—and the steps they can take to dramatically lower that risk. The campaign emphasizes managing not only blood glucose (best measured by the A1C test), but also blood pressure, and cholesterol.

Working with its many partners and community contacts, the NDEP hopes to close the gap between what is known about the best diabetes treatments and what is actually practiced at doctors' offices and health clinics throughout the U.S. Ultimately, the NDEP aims to reduce the illness and deaths associated with diabetes and its complications.

PATIENT PROFILE

Monica Boone

A Patient in the Diabetes Prevention Program

Monica Boone, a soft-spoken mother of two from Zuni, New Mexico, was haunted by a fear of diabetes. Like many other Native Americans, several members of her family—of the Zuni Indians in New Mexico—had been stricken by type 2 diabetes. It had killed her father, who died of a diabetes-induced heart attack at age 57, and one of his brothers. Another paternal uncle now struggles with the disease. She thought there was a good chance she was next in line. Three years ago, Monica, who is 5-feet 3-inches tall, weighed 173 pounds, had little energy, and didn't get much physical activity. So, she became interested in a diabetes research study that was just beginning in Zuni. She was permitted to participate in the study when her blood tests revealed that she had pre-diabetes, a condition just one step away from outright diabetes.

Pre-diabetes affects about 16 million people in the U.S. Individuals with pre-diabetes have blood glucose levels that are higher than normal, but not yet in the range that indicates diabetes. Having pre-diabetes sharply increases the risk of developing type 2 diabetes and heart disease. Once a person develops type 2 diabetes, the risk of heart disease is even higher—two-to-four-times that of people without diabetes.

"I was scared for myself and my family, but I still wanted to know where I stood," she recalls. "When I found out about the study, I felt I was being given a second chance. I decided to take that chance. I wanted researchers to find out if this disease can be prevented."

Monica Boone was a participant in the Diabetes Prevention Program (DPP) clinical trial. She helped demonstrate that improvements in diet, coupled with moderate exercise, can delay and possibly prevent type 2 diabetes in those at high risk for the disease. Photo: Indian Health Service.

MONICA BOONE

Like thousands of other adults across the country at high risk for type 2 diabetes, Monica joined a research study called the Diabetes Prevention Program (DPP), a large, multi-center clinical trial spearheaded by the NIDDK. All of those enrolled in the study had pre-diabetes and were overweight. Participants were randomly assigned to one of three groups:

Lifestyle Changes: Participants in this group aimed to lower their body weight by 7 percent by reducing their intake of fat and calories, and by exercising 150 minutes a week with moderate intensity. Most chose walking an average of 30 minutes a day, 5 days a week.

Drug Treatment: Participants in this group took 850 milligrams of the oral diabetes drug metformin twice a day. This group also was given standard information on diet and exercise. Metformin lowers blood glucose mainly by decreasing the liver's production of glucose.

Placebo: Participants in this group took placebo pills in place of metformin. This group also received standard information on diet and exercise.

Monica was randomly assigned to the group focused on lifestyle changes. She worked to lose 7 percent of her weight—about 12 pounds—by curbing fat and calories in her diet, and by exercising 150 minutes per week. She decided her exercise would be to walk. Her first time out was tough. "My heart was beating so fast," she recalls. Slowly, she built up her endurance from 1 to 4 miles, at least 5 days a week. She gradually mixed in jogging with walking. She ate less "fast food" and began cooking nutritious meals at home. Her weight started to drop. In time, she lost 20 pounds. Best of all, her blood glucose levels returned to normal.

Monica was one of the 3,234 DPP participants who helped demonstrate that improvements in diet coupled with moderate exercise can delay and possibly prevent type 2 diabetes. Specifically, diet and exercise resulting in a 5-to-7 percent weight loss lowered the development of type 2 diabetes by 58 percent in this high-risk group. The study also found that metformin reduced the risk of developing type 2 diabetes by 31 percent. The drug was most effective in younger, heavier individuals.

The good news didn't end there. While both interventions lowered fasting blood glucose levels, diet and exercise were more effective at lowering glucose levels 2 hours after a standardized glucose drink—the "oral glucose tolerance test." Also, about twice as many people in the lifestyle group, compared to those who received standard information, regained normal blood glucose levels, showing that diet and exercise can reverse the pre-diabetes that often leads to type 2 diabetes.

These findings show that people don't have to exercise excessively or starve themselves to lose weight in order to achieve the goal of preventing type 2 diabetes. Dr. Rena Wing, a Brown University professor who oversaw the lifestyle portion of the study, adds that, "We're not saying to people that they need to achieve ideal body weight. These are reasonable goals." Indeed, the study participants lost, on average, a modest 15 pounds.

MONICA BOONE

Extensive NIDDK and NIH-supported advances in clinical research on obesity, nutrition, and behavior converged in the design of this clinical trial, particularly the intensive lifestyle intervention arm. By employing counseling methods and information on diet and exercise that had previously proven most effective, the researchers were able to help participants achieve their weight loss goals. This provided the researchers with a large enough pool of successful participants to satisfactorily answer the question, "Can diabetes be delayed or prevented through lifestyle changes?"

Researchers who conducted the DPP study announced their results in August 2001, at a press conference convened by Health and Human Services Secretary Tommy G. Thompson. They concluded that the findings of the study were so dramatic and had such great potential for stopping or delaying the onset of new cases of type 2 diabetes, that the study should be terminated a year sooner than planned. Their findings were reported on February 7, 2002, in the *New England Journal of Medicine*.

Other research has shown that diet and exercise can delay type 2 diabetes in at risk people. But the DPP, conducted at 27 centers nationwide, is the first major study to show that lifestyle changes can delay diabetes in a diverse population of overweight American adults with pre-diabetes. Nearly one-half of the DPP participants were from minority groups that suffer disproportionately from type 2 diabetes: American Indians, African Americans, Hispanic Americans, Asian Americans, and Pacific Islanders. Diabetes has hit American Indians harder than any other ethnic group in the U.S., taking an enormous toll in pain, disability, and loss of life. On average, American Indians and Alaska Natives are 2.6 times more likely to have diabetes than non-Hispanic Caucasians of similar age.

Can lifestyle changes or metformin treatment prevent diabetes completely? "We just don't know how long diabetes onset can be delayed, beyond the 3-year period studied," says Dr. David Nathan, of the Massachusetts General Hospital—the Chairman of the DPP. "We hope to follow the DPP volunteers to learn how long the interventions are effective." The researchers will analyze the data to determine whether the interventions reduce heart disease and atherosclerosis, major causes of death in people with type 2 diabetes.

Behind Monica Boone's house are the trails she runs and has come to love, paths that wind through a valley surrounded by the stark beauty of Corn Mountain and the Bluebird Mesas. Since joining the DPP study, she has gone through 10 pairs of running shoes. "I look forward to my runs now," she says. "I see small animals like rabbits and rodents and beautiful birds, even a golden eagle sometimes. I have more energy; I'm quicker in my movements; and I enjoy going here and there. I used to dread it. People say, 'Is that you?' They don't recognize me," she laughs.

PATIENT PROFILE

Aylin Riedel

Management of Diabetes and Kidney Disease as a Daily Balancing Act

Most of us seek balance in our lives. But for 30-year-old Aylin Riedel, a person with type 1 diabetes whose condition has led to kidney disease, medically referred to as nephropathy, the pursuit of balance takes on a whole new meaning. Starting at age four-and-one-half, when she was diagnosed with diabetes, Aylin has worked hard to balance her glucose levels on a daily basis to keep her diabetes under control. Despite her best efforts, about 8 years ago she began experiencing higher blood pressure and kidney problems related to her diabetes, so now she also struggles to keep her blood pressure in check. Her situation is compounded by the fact that her body responds poorly to the angiotensin-converting enzyme (ACE) inhibitor she needs to control her blood pressure and retard her kidney disease. This has exacerbated a condition called orthostatic hypotension that makes Aylin dizzy and causes her to literally lose balance frequently when she stands up from a sitting or lying position. Without the ACE inhibitor, however, she faces the real possibility of having to go on dialysis or receive a kidney transplant within a couple of years.

Pictured are Aylin (left) and her husband, Eric (right). Nearly every decision that Aylin makes needs to be weighed against her diabetic condition and subsequent kidney disease. Since she began using an insulin pump two years ago, she enjoys more freedom.

Impact on Life Decisions

Aylin's need to achieve balance in her life doesn't stop with her blood sugar, blood pressure, or medications. Nearly every decision she makes needs to be weighed against her diabetic condition and subsequent kidney disease. "For example, I could never be self-employed or work for a company that didn't provide adequate health insurance as a benefit to its employees," says Aylin, who holds a Ph.D. in health care economics and works for a large managed care organization.

Conservative estimates place the total cost of diabetes in the U.S. at $98 billion annually, including direct medical costs and costs associated with disability, work loss, and premature mortality.

AYLIN RIEDEL

Aylin's health also has implications for people she loves dearly. One of the most difficult decisions Aylin and her husband, Eric, recently have had to make was to weigh the trade-off between having their own biological child and the fact that pregnancy could possibly hasten the deterioration of Aylin's kidneys. Women who manifest nephropathy before pregnancy run as high as a 90 percent risk of developing hypertension and pre-eclampsia, a condition that can cause dangerously high blood pressure in the mother and force an early delivery of the baby. After consulting with two physicians, Aylin and Eric decided against pregnancy. "It was a shock to us...we had always expected that we would have biologic children," Aylin says. "Now we are exploring other ways to build our family." She and Eric are pursuing the possibility of adopting a child from overseas, which unfortunately comes with its own set of issues for the couple. "We've learned that overseas adoption agencies take into consideration the health of the prospective adoptive parents, including life expectancy," says Aylin, almost matter-of-factly. The fact is that kidney disease is a major cause of excess illness and premature death in people with type 1 diabetes.

The Impact of Research

Aylin uses an insulin pump to provide her daily insulin requirements. "I've been using the pump for 2 years, and I think it's the greatest invention for diabetes," she says. "It gives me so much more freedom than having to take insulin shots."

The good news is that an extensive body of research aimed at understanding the underlying mechanisms of both diabetes and kidney disease is well under way, much of it funded by the NIDDK. The goal is to develop effective treatments and possible methods of prevention.

Prior to taking an ACE inhibitor, Aylin's blood pressure at times would spike to 220 over 140. The ACE inhibitor Aylin now takes, however, induces serious side effects that affect her balance. It is hoped that one or more of the medications currently being developed to enhance blood pressure control, used in combination with an ACE inhibitor, may reduce the side effects in patients like Aylin.

New Blood Pressure Medications—NIDDK-supported research has established the value of a specific type of drug, ACE inhibitors, and specific blood pressure targets in slowing progress of kidney disease. These measures are helping patients preserve kidney function while controlling their blood pressure.

Low-Protein Diets—Researchers are finding that a diet containing reduced amounts of protein may benefit people with kidney disease. Therefore, experts are recommending that most patients with advanced nephropathy consume limited amounts of protein.

Intensive Management of Blood Glucose—Major NIDDK-supported studies in type 1 and type 2 diabetes provide compelling evidence that keeping blood glucose as close to normal as possible dramatically reduces onset and progression of diabetic kidney disease. The regimen includes frequently testing blood glucose, administering insulin frequently throughout the day on the basis of food intake and exercise, following a diet and exercise plan, and frequently consulting a health care team.

AYLIN RIEDEL

Genetic Research—In addition to new medications, diet, and intensive glucose management, researchers also are investigating the genetic links to diabetes and kidney disease. For example, recent research sponsored by the NIDDK has identified a "variation" in the apolipoprotein E gene that, in type 1 diabetics, is associated with a three-fold greater risk of developing kidney disease. NIDDK-supported researchers are hoping to find more genetic relationships like this one through an ongoing large-scale study of families with diabetic kidney disease.

Much remains unknown when it comes to diabetes and its impact on major organs. Although we can slow development of diabetic kidney disease, we cannot prevent it. Also, it is still unknown why some people are more genetically predisposed to diabetes and kidney disease than others.

Living with the Disease

In many respects, Aylin is very fortunate. "Without ACE-inhibitor treatment, Aylin would very likely have experienced renal failure at this point," says her personal physician, Betsy Seaquist, M.D., who conducts diabetes research at the University of Minnesota. "However," she adds, "complications never exist in a vacuum, and treatment for the nephropathy has caused Aylin serious side effects. In addition to the orthostatic hypotension, which causes Aylin to lose her balance, she suffers from cardiac problems and damage to her eyes," says Dr. Seaquist.

"Although my vision is now stable," says Aylin, "I've undergone lots of laser surgeries." Nonetheless, her diabetic condition has left her with less than acute vision in one of her eyes. Consequently, Aylin, whose work as an economist entails lots of reading and writing, is forced to use large fonts on her computer. "I'm very up-front with my employers about my diabetes and how it intersects with my work life," says Aylin. "Before I am even hired, I tell them that I need special accommodations, including more time off for doctors' appointments. I get away with it because I'm very good at what I do," she adds.

It's not unusual for people with diabetes to see a number of medical specialists, including ophthalmologists for eye examinations, podiatrists for routine foot care, dieticians for help in planning meals, and diabetic educators for instruction in day-to-day care.

Aylin would like nothing more than to do away with her daily balancing act. "I'd like to think that a pancreatic/kidney transplant would cure me of my diabetes," she says. The irony is that, given her intolerance to the ACE inhibitor she now takes, she still would need to weigh the transplant against the impact that the immunosuppressant drugs, required to avert rejection of the transplanted organ, would have on her body for the rest of her life.

Although current therapies have done much to delay the need for dialysis or organ transplants in people with diabetes who suffer from nephropathy, much more still needs to be done. The lives of Aylin Riedel and millions of others hang in the balance.

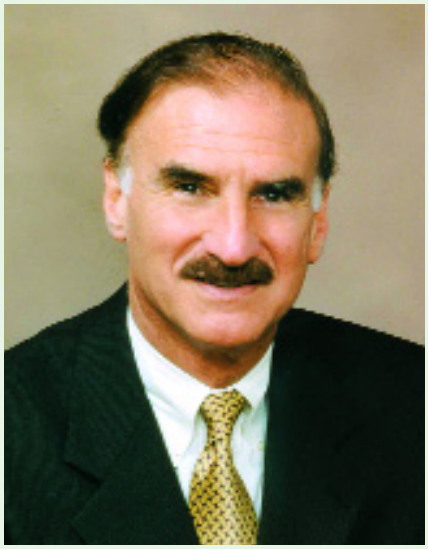
The prevention and treatment of the long-term micro- and macrovascular complications of diabetes—kidney, eye, nerve, and cardiovascular disease—remain critical problems in diabetes care. Because the blood vessel damage leading to these complications can begin as soon as a person becomes diabetic, early intervention is key. The NIDDK is currently spearheading efforts to identify "surrogate markers" for the micro- and macrovascular complications of diabetes. These surrogate markers would indicate disease progression before it is clinically apparent. The hope is that such surrogate markers will assist researchers in identifying individuals at risk for developing diabetes-related complications, and also enable them to evaluate the benefits of current and evolving therapies.

VISION STATEMENT

C. Ronald Kahn, M.D.

A Vision of the Future: Diabetes Research

The NIDDK National Advisory Council meets three times annually to provide advice to the Institute regarding its research portfolio and broad issues of science policy. The Council members are an important liaison between the research communities they represent and the NIDDK, which supports each community's research efforts. The "Vision Statements" included in this year's "NIDDK: Recent Advances and Emerging Opportunities" are meant to capture the essence of five scientific presentations given by outgoing Council members in 2002. These presentations were opportunities for experts in the fields of diabetes, digestive diseases, behavioral intervention and health, urology, and kidney disease to reflect on important basic and clinical research accomplishments and what may—or should—be part of future endeavors.



Dr. C. Ronald Kahn

Dr. C. Ronald Kahn is the President of the Joslin Diabetes Center and the Mary K. Iacocca Professor of Medicine at Harvard Medical School, Boston. Dr. Kahn's main research interest is in type 2 diabetes, specifically insulin action and insulin receptor biology, including creation and use of insulin receptor knockout mouse models.

In 1998, Dr. Kahn was named chairman of the congressionally-established Diabetes Research Working Group (DRWG). The research opportunities and needs identified in the DRWG's Strategic Plan, issued in 1999, were the culmination of a year-long planning process led by sixteen members, non-governmental experts in the diabetes field in consultation with other leading scientists. Since 1999, the DRWG's Strategic Plan has served as a major scientific guidepost to the NIH for diabetes research. In 2002, the NIH issued a scientific progress report on the DRWG's Strategic Plan, entitled "Conquering Diabetes: Highlights of Program Efforts, Research Advances and Opportunities." (http://www.niddk. nih.gov/federal/advances/advances_02.html)

The DRWG's Strategic Plan identified five areas of diabetes research in which the current rapid expansion of knowledge and development of new technologies made it likely that intensified research efforts would lead to significant advances in the near future. These areas of "extraordinary opportunity" included: the genetics of diabetes and its complications, autoimmunity and the beta cell, cell signaling and cellular regulation, obesity—a major risk factor for type 2 diabetes—and clinical research and clinical trials of critical importance. At the September 2002 meeting of the National Diabetes, Digestive, and Kidney Diseases Advisory Council, Dr. Kahn highlighted progress in diabetes research since the 1999 issuance of the DRWG's Strategic Plan.

VISION STATEMENT

Genetics of Diabetes and Its Complications and Obesity

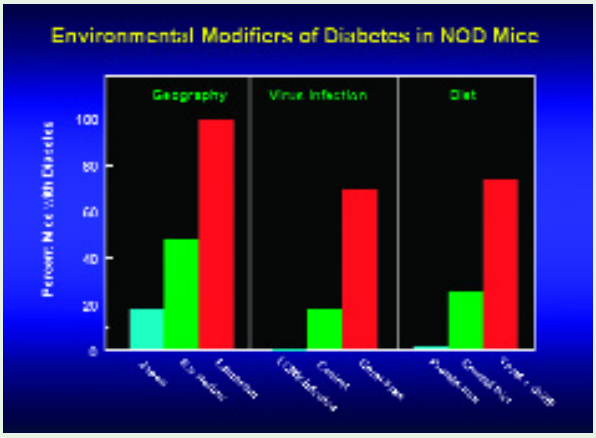
Both type 1 and type 2 diabetes are complex genetic diseases that result from interactions between multiple genes and environmental factors. Approximately 80 percent of the type 2 diabetes in the U.S. occurs in overweight or obese individuals. Real progress has been made in the area of the genetics of diabetes and its complications. For example, in type 1 diabetes, researchers identified an important gene cluster—the major histocompatibility genes in the HLA locus. Research efforts on type 2 diabetes and obesity led to the identification of six genes contributing to Maturity Onset Diabetes of the Young (MODY); six monogenic forms of insulin resistance and/or obesity; and two genes for lipoatrophic diabetes. However, it has proven more difficult to identify genes responsible for type 2 diabetes, the most common form of the disease. Multiple candidate genes have been studied and some, such as the *calpain-10* gene (see main text), have received considerable attention, but clearly this is not the major type 2 diabetes gene in humans, so much work needs to be done in this area. The same is true for the genetics of the complications of diabetes. A number of candidate genes have been studied, and certainly there is an important genetic risk for kidney complications of diabetes; however, the genes responsible have not yet been identified. Major initiatives to discover the genetic basis of obesity have also begun to uncover genes that may determine susceptibility not only to obesity but also to the subsequent development of type 2 diabetes. The identification and understanding of the many genetic determinants of both forms of diabetes, their risk factors, and the complications that they share are of critical importance to conquering this disease. Dr. Kahn encouraged the NIDDK to enhance research to define genes responsible for type 2 diabetes and obesity by strengthening existing consortia. Additional efforts should also focus on genome-wide screens in humans to search for genetic changes that may play a role in increased risk of diabetes and obesity. The newly-initiated Diabetes Genome Anatomy Project may provide important clues about how genetic variation predisposes to disease development in both animal and human models.

By way of example, Dr. Kahn highlighted some of his own research. His laboratory developed a polygenic model of type 2 diabetes by creating "knockout" mice that were 50 percent deficient in the insulin receptor and in its major substrate, IRS-1. The degree of diabetes in these mice greatly depended on their original genetic background, varying from zero to over 90 percent. This finding signifies the challenge of human type 2 diabetes by demonstrating that any genetic predisposition to development of the disease can be modified by other genes. A genome-wide scan would provide the best chance of finding these "modifier" genes and determining their effect on the development of diabetes.

Environmental factors play a role in the development of obesity, which correlates with the development of type 2 diabetes. However, there is wide variability in research data. While excess calorie consumption, lack of physical activity, and overweight and obesity are clearly major contributors to the increasing prevalence of diabetes, these may not be the only environmental factors. Of note, the relative proportions of those with overweight or obesity and diabetes vary among regions, indicating that here may be other environmental modifiers that may make certain individuals or populations more likely to develop type 2 diabetes. Dr. Kahn stated, "I would like to caution us not to simply assume that the more we eat and the fatter we get, the more diabetes we have. There could be other environmental modifiers that we're overlooking because obesity is an obvious modifier."

VISION STATEMENT

The environment is an important modifier in the genetic predisposition to development of type 1 diabetes. The best-known mouse model of type 1 diabetes, the non-obese diabetic (NOD) mouse, is a genetically bred strain in which every mouse is identical. Researchers have shown that in a germ-free environment, approximately 80 percent of these mice develop diabetes. In contrast, the rate of diabetes development drops dramatically when the mice are exposed to certain infectious pathogens. Diet can also affect the development of diabetes. Environmental modifiers may thus provide important targets for disease prevention and treatment.



Environmental modifiers play a major role in the development of diabetes. This chart shows the prevalence of diabetes in the non-obese diabetic mouse (NOD mouse), a genetically inbred mouse model of diabetes. The percentage of mice with diabetes varies from less than 5 percent to almost 100 percent depending on geography, exposure or lack of exposure to certain viruses, and modifications in diet.

Autoimmunity and the Beta Cell

Type 1 diabetes is an autoimmune disease in which the immune system attacks the insulin-producing beta cells of the pancreas. The beta cell is also central to the development of type 2 diabetes because it must meet the increased requirements for insulin during the period of insulin resistance that typically precedes disease onset. Ultimately, the beta cell begins to fail under this strain, leading to type 2 diabetes.

Over the past five years, tremendous progress has been made in preventing the rejection of islet transplants, in the understanding of basic mechanisms of autoimmunity, and in beta cell development and regulation. Additional work, however, is needed in the prevention of type 1 diabetes. Dietary manipulations, immunosuppressive regimens and cellular treatments have all been successful in modifying the course of type 1 diabetes in animal models. Although the insulin injection arm of the Diabetes Prevention Trial for Type 1 Diabetes did not reduce the development of diabetes in those at high risk, we learned a great deal about how to predict diabetes that will prove useful in future trials. We are awaiting results of the oral insulin arm which still holds great promise in the prevention of the disease. Dr. Kahn said, "I think that the prevention of type 1 diabetes should be a high priority at the level of human clinical investigation. This is an important way to stop the problem of type 1 diabetes so that ultimately islet cell transplantation will not be necessary." In addition, further efforts should focus on postnatal beta cell development to match research efforts on embryonic beta cell development. Advances in this area may lead to the ability to regenerate islets in the future.

Cell Signaling and Cell Regulation

Cells throughout the body signal to each other to coordinate vital functions, such as maintaining blood glucose concentration within a narrow range and holding body weight at steady levels. A breakdown in this highly integrated communication network, or in the signaling and response pathways within a cell, can lead to diabetes, obesity, and diabetes-associated complications.

VISION STATEMENT

A key signaling molecule is the hormone insulin. Secreted by the beta cells of the pancreas, insulin travels throughout the body to other organs and tissues to regulate blood glucose levels and to influence a variety of other cellular processes. Great strides have been made in unraveling insulin signaling and new knowledge is accumulating about this complex network. Insulin resistance, the body's manifestation of abnormal insulin signaling, produces a wide range of phenotypes, depending both on the specific tissue that is resistant to the action of insulin and the point in the insulin signaling pathway that is abnormal. Mice with altered insulin signaling in the beta cell lose the ability to secrete insulin in response to glucose, have progressively impaired glucose tolerance, and a decrease in the growth of pancreatic beta cells. In the brain, insulin plays a role in the regulation of appetite, and in the development of obesity and insulin resistance. Mice lacking the insulin receptor in the lining of blood vessels are partially protected from the development of diabetic complications, such as diabetic eye disease. This finding seems to indicate that insulin itself may play a role in the development of the devastating complications of diabetes.

This research also demonstrates that hormone action goes beyond the "classic" target tissues. Tissues have the ability to "talk" to each other through the release of chemical signals. For example, the fat cell, through the release of many different chemicals, can affect insulin sensitivity and insulin resistance in muscle and other tissues far removed from the fat cells.

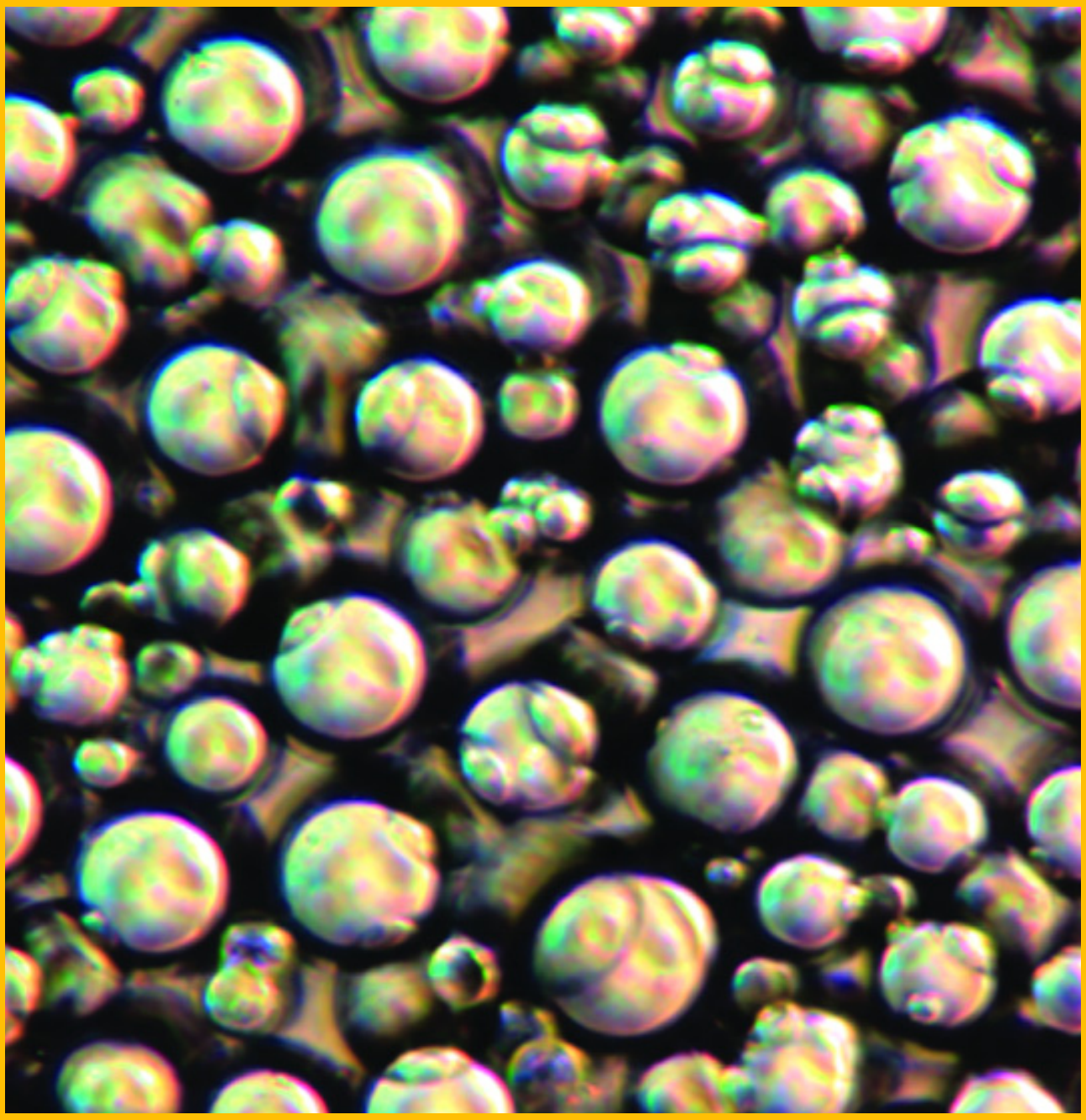
Cellular signaling involves many complex pathways and is central to insulin action, immunological function, and to understanding appetite regulation in the brain—all important factors in the development of both type 1 and type 2 diabetes. Continued study of the body's coordinated regulation of cell signaling is vital to further understanding in diabetes and metabolism.

Clinical Research and Clinical Trials of Critical Importance

The National Diabetes, Digestive, and Kidney Diseases Advisory Council has long been concerned with clinical research. The DRWG emphasized the importance of a substantial investment in clinical trials and clinical research to validate in humans the fundamental observations made in the laboratory and to permit testing of therapeutic strategies. Since the issuance of the DRWG's Strategic Plan, the NIDDK has significantly expanded clinical research directed at advancing the prevention and care of diabetes. In particular, considerable work has been done to establish the clinical infrastructure needed to efficiently conduct large, long-term trials by creating national, multi-center research networks or consortia. For example, the TrialNet for type 1 diabetes will perform intervention studies to preserve pancreatic beta cell function in patients with new-onset type 1 diabetes, and to prevent type 1 diabetes in high risk individuals. However, critical needs still exist for more clinical investigators and improved training programs. It is vitally important to further expand and improve training of clinicians and to bring more clinical investigators into diabetes research in order to realize a real decline in the prevalence of the disease.

Challenges for the Future in Diabetes Research

Following on an impressive record of accomplishments in all these areas of diabetes research, Dr. Kahn sees many challenges remaining for the future. The NIDDK will play a vital role in meeting these challenges—both through support of research ideas and support of the human resources and technologies that make research possible. Dr. Kahn concluded his presentation by stating, "I would like us to continue to evaluate our human resource needs. I think the pipeline of new investigators is not as robust as I would like it to be. I also believe that we still need to push ourselves to look at the next generation of technologies that will become available."



These fat cells were isolated using a method developed by Nobel laureate Dr. Martin Rodbell, whose prizewinning work was supported by NIDDK. In humans, fat cells located in the abdomen secrete hormones believed to be responsible, at least in part, for insulin resistance. Photo: Dr. Joseph Brzostowski and Ms. Mary-Jane Zarnowski, NIDDK.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. They include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. NIDDK-funded scientists are vigorously pursuing research to understand the causes of these diseases and how they progress, and to test pharmacological, surgical, and behavioral interventions for treatment and prevention.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. Overweight and obesity also disproportionately affect minority populations, particularly African American, Hispanic, and Native American women and children. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacological agents to promote safe, long-term weight loss. Investigators are also continuing behavioral research to help people achieve lifestyle modifications that include increased physical activity and improved diet.

Numerous liver diseases have serious adverse impacts on health and can progress to the need for a liver transplant for survival. Scientists are intensifying research on a variety of liver diseases, from those primarily affecting children, such as biliary atresia, to those commonly affecting adults, such as nonalcoholic steatohepatitis; and from those caused by infection, such as hepatitis C, to those resulting from a variety of other factors, such as autoimmune reactions, genetic mutations, drug toxicity, and as-yet-unknown triggers. Among ongoing and planned clinical research efforts are investigations of treatments for hepatitis C infection and other liver diseases, and studies related to liver transplantation.

The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. Surgical treatment is often required. Scientists are dissecting the complex interactions among the genetic and environmental factors that contribute to the development of IBD. The continued identification of predisposing genetic variations and other factors, such as potential autoimmune and microbial influences, will help spur the design of novel therapeutic strategies.

Another intestinal disorder, irritable bowel syndrome, causes pain and constipation or diarrhea and is especially common in women. While diet and stress contribute to this disorder, the underlying causes are unknown. Symptoms may be influenced by abnormal functioning of the intestinal nervous system and altered perception of intestinal stimuli by the brain. Scientists are accelerating research to better understand and treat this disorder.

Digestive disease can also be triggered by foods. In individuals with celiac disease, the immune system reacts to a protein called gluten, which is a component of wheat, barley, and rye. This reaction leads to damage to the small intestine, interfering with its ability to absorb nutrients from foods. Celiac disease is associated with a variety of conditions, such as abdominal pain, anemia, osteoporosis, and increased risk of cancer. Following a gluten-free diet is difficult but currently the only treatment; continued scientific research is expected to lead to new therapies.

Among other diseases of the digestive tract are those of the pancreas, including various forms of pancreatitis. Chronic pancreatitis, serious in and of itself, also confers increased risk for pancreatic cancer, one of the deadliest malignancies. Scientists are identifying both genetic and environmental factors associated with pancreatic disease and pancreatic cancer.

Finally, microbes affect digestive health in powerful and sometimes surprising ways. Many foodborne illnesses are caused by bacteria, such as certain strains of *E. coli*, and ulcers and stomach cancer are linked to *H. pylori* infection. Scientists are also gaining insights into how the microorganisms that normally reside in the gut influence the development and function of the digestive tract.

FINDING WAYS TO UNDERSTAND AND TREAT OBESITY

Obesity has emerged as one of the greatest threats to human health and well-being, with the number of those affected continuing to escalate at an alarming rate. Several ways of measuring overweight or obesity exist, including Body Mass Index, or BMI. BMI is a ratio derived from a person's weight and height; people with a BMI of 25 or greater are considered overweight, while those with a BMI 30 or greater are classified as obese. By these measurements, according to the Centers for Disease Control and Prevention, overweight and obesity affect an estimated 64 percent of the U.S. adult population, with serious consequences for the health of the nation. Furthermore, 15 percent of children and teens are also overweight. These individuals are at increased risk for coronary heart disease, diabetes, stroke, and some forms of cancer. Furthermore, data analysis in a recent study suggests that obesity, particularly in young adults, markedly reduces life expectancy.

Obesity occurs when the balance of food intake and energy expenditure is perturbed; excess energy is stored mostly as fat. Maintaining the "energy balance" depends on complicated interactions of many biological and behavioral factors, including genetic predisposition, food intake, and activity level. Although vigorous research has uncovered genes and metabolic pathways that contribute to obesity, a cure remains elusive. Thus, scientists continue to seek answers and look for novel approaches to find means to treat and prevent obesity.

Investigating Leptin's Effects on Metabolism: A significant milestone in our understanding of obesity was the discovery of the fat-cell-derived hormone, leptin. After a meal, fat cells release leptin. The hormone travels to the "appetite-control center" in the hypothalamus, where it binds to leptin receptors (molecules that "see" leptin) on specialized brain cells and signals the brain to "stop!" eating. Scientists found that mice lacking the gene for leptin—*ob/ob* mice—overeat and become obese. When given leptin, these mice lose weight. Unfortunately, administering leptin to humans (except for the few identified with leptin deficiency) does not effectively treat obesity; in fact, most obese humans have high levels of the hormone, indicating that they are resistant to its effects.

However, in addition to acting to repress appetite, leptin also elicits metabolic changes in numerous tissues in order to maintain weight homeostasis. These metabolic changes include increased fat burning (oxidation) and increased glucose uptake. How leptin exerts these effects is still not completely understood. In recent studies, NIDDK-supported scientists set out to determine the effects of leptin in various tissues and the molecular mechanisms and cell signaling pathways involved.

In one study, NIDDK-supported researchers compared the effects of making specific tissues "blind" to leptin. They generated two different groups of mice through genetic engineering, one severely deficient in brain cell leptin receptors and the other severely deficient in liver cell leptin receptors. They found that mice deficient in brain cell receptors for leptin became obese and had enlarged, fatty livers. In contrast, mice deficient in liver cell leptin receptors were lean and appeared to have normal livers. These studies demonstrate that disruption

of the leptin receptor in just the brain can recreate the characteristics of overall leptin deficiency as observed in the *ob/ob* mouse—strongly suggesting that the effects of leptin deficiency on the liver are due to downstream effects of defective leptin signaling in the brain, rather than loss of leptin signaling in the liver itself.

In another study, NIDDK-supported researchers used a technique known as transcription profiling (with microarrays) to identify genes regulated by leptin in the liver of mice. They found that leptin represses the gene that encodes the enzyme SCD-1, decreasing its activity levels. This enzyme catalyzes the synthesis of monounsaturated fatty acids. In order to more fully understand the effect that this enzyme has on obesity, the researchers also studied a mouse strain lacking a functional SCD-1 gene. They observed that these mice are lean, with a higher than normal metabolic rate. The team then used this strain in crosses (matings) to generate a mouse strain that was both SCD-1 deficient and leptin-deficient. They found that leptin-deficient *ob/ob* mice lacking SCD-1 were less obese and had an increased metabolic rate compared to leptin-deficient *ob/ob* mice with a normal SCD-1 gene. Thus, a potential mechanism for leptin's impact on body weight is its inhibition of SCD-1, which may result in a decrease in the synthesis of fat and an increase in its oxidation.

These new studies in mouse models have advanced scientists' understanding of leptin's effects on obesity. They have demonstrated that leptin must bind to receptors on brain cells to have generalized effects on obesity, and that the enzyme SCD-1 is responsive to leptin levels and may be a potential new target for drug intervention in the treatment of human obesity.

Signaling Cells to Burn Energy—An Important Part of Energy Balance: Multiple lines of evidence have led to the current model of energy balance that dictates that, as the brain receives signals of excess calorie intake, it responds by both decreasing appetite and increasing energy expenditure in order to prevent excessive weight gain. The latter phenomenon is known as "diet-induced thermogenesis." Diet-induced thermogenesis is thought to be mediated by the sympathetic nervous system (SNS), *via* stimulation of the beta-adrenergic receptors located on target cells—particularly cells that can "burn off" stored energy in fat by releasing it as heat, rather than using it to power other chemical reactions. When calories are lost as heat energy, they are removed from the total energy balance in the body. However, this hypothesis regarding diet-induced thermogenesis has never been directly tested.

To test this hypothesis, NIDDK-supported researchers generated mice without any active beta-adrenergic receptors and compared them to normal mice. They found that the test mice had a lower metabolic rate and were somewhat fatter than the normal mice when they were fed a typical chow diet and became massively obese when they were fed a high-fat diet. The researchers were also able to demonstrate that the obesity that developed in mice without these receptors was the result of a lower metabolic rate and was not due to a decrease in activity or to an increase in food intake. Thus, beta-adrenergic receptor mediated signaling pathways are indeed necessary for diet-induced thermogenesis, and may represent a possible therapeutic target for preventing or treating obesity.

A Genetic Locus for Severe Obesity: While sedentary lifestyles and unhealthy diets contribute to obesity, heredity also plays a role. However, the search for predisposing genes has been hampered by the genetic complexity of obesity—no single gene is responsible for all human weight gain. Now, in a collaboration between academia and industry, a team of investigators has found a chromosomal region (locus) linked to severe obesity in females.

To design a genetic hunt to circumvent some of the genetic complexity of obesity, the scientists focused on very severely obese individuals. The more extreme a disease, the stronger the genetic influence is likely to be, and the greater the likelihood that it can be pinpointed. They also studied families with very obese members who were closely

related, and thus likely to share the same predisposing genetic variation. Additionally, the scientists incorporated strategies to detect potential gender-specific effects. With DNA samples from hundreds of people and sophisticated computer programs, the scientists pinpointed a locus on chromosome 4 that harbors a gene strongly linked to obesity in females. It is not yet clear whether it affects males. The industrial partner in the collaboration recently announced identification of the predisposing gene within this locus, which they term HOB1. Details about the HOB1 gene have not yet been published in a scientific journal as this document goes to press. However, it is anticipated that understanding how the gene functions could lead to drug development to modulate its effects on obesity.

Exploring Novel Compounds That May Lead to New Obesity Treatments: As indicated by the preceding studies, new opportunities to develop effective, innovative treatments for obesity are emerging from a better understanding of normal weight regulation and how it is disrupted in obesity. Importantly, such studies are complemented by continued experimentation with novel compounds.

One recent research study explored the potential for using a molecule that mimics insulin to control weight. Insulin is a pancreatic hormone that, in addition to stimulating glucose uptake by body cells, interacts with receptors on brain cells in the hypothalamus to modulate energy balance. Through a set of experiments in animals, researchers had already shown that insulin injected into the brain reduces food intake and body weight. In contrast, insulin administered systemically has no effect on curbing obesity, and actually leads to weight gain. An NIDDK-supported research team recently carried out similar experiments, using small molecules that mimic insulin (insulin "mimetics"). When administered to the brain of rodents, an insulin mimetic had the same weight-reducing effect as insulin, but when an insulin mimetic was given orally to rodents, it did not lead to weight gain (as systemic insulin does) and, in fact, reduced diet-induced obesity in these animals. The ability of a small molecule insulin mimetic to control weight when administered orally may give it a significant advantage over natural insulin in terms of its impact on body weight.

Drugs that increase serotonin activity in the brain are frequently used to assist in weight loss, because they suppress appetite. In the mid 1990s, one such drug, fenfluramine, often given in combination with phentermine, was widely prescribed as a weight loss therapy. Although it was prescribed to millions for weight loss because it decreases food intake, fenfluramine and a related drug, dexfenfluramine, were removed from the U.S. market by the FDA due to reports that they caused high blood pressure in the lungs and heart valve damage. Researchers recently studied the activity of dexfenfluramine (d-FEN) in rodents to determine the exact mechanism by which it reduces appetite. Their studies demonstrate that d-FEN stimulates a specific pathway in the brain's appetite control center, the "central melanocortin system," a fundamental regulator of food intake and body weight in rodents and humans. This effect appears to be initiated through the activation of serotonin receptors on certain brain cells, POMC neurons, that are part of the central melanocortin system. Researchers may now be able to develop drugs that act along this pathway in a manner similar to d-FEN, but without producing the damaging side effects that d-FEN and fenfluramine produce.

In other studies, scientists looked at a synthetic compound, C75, which is known to reduce appetite and body weight in mice. In an obese mouse model and normal control mice, a single dose of C75 caused reductions in food intake and body weight. However, when the mice were given lower doses of C75 over a longer time period, the normal mice initially reduced their food intake but became tolerant to C75 after the first day: their food intake returned to near normal and no additional weight was lost. In contrast, the obese mice continued to eat less and lose weight throughout the five-day trial. When researchers gave another group of control mice the same quantity of food as consumed by the C75-treated mice, they lost 25 to 50 percent less

weight than the C75-treated mice. This discrepancy indicates that, in addition to suppressing appetite, C75 may stimulate an increase in the metabolic rate that accounts for the extra weight loss in the C75-treated mice. Future studies may elucidate whether C75 or related compounds will be useful for treating human obesity.

NIDDK Efforts in Obesity: These basic and pre-clinical research studies illustrate the multiple approaches researchers are taking to understand and address weight regulation and obesity at the cellular and molecular level. The hope is that the knowledge gained from these studies will accelerate success in clinical interventions to combat obesity.

To fuel these studies, the NIDDK maintains a strong program of research on and related to obesity, both as a serious risk factor for type 2 diabetes and its complications and as an independent health problem. The National Task Force on the Prevention and Treatment of Obesity was established by the NIDDK and includes both NIH scientists and experts from the extramural community. This Task Force provides science-based guidance to aid research strategies. The Task Force also generates public health messages about obesity. The NIDDK also supports Obesity/Nutrition Research Centers and Clinical Nutrition Research Units, which conduct both basic and clinical research studies.

With support from other NIH Institutes, Centers, and Offices, the NIDDK has launched a multi-center clinical trial that will examine the health effects of intentional weight loss in obese diabetic patients, with particular emphasis on cardiovascular health. The trial is called Look AHEAD (Action for Health in Diabetes). In collaboration with a number of other Institutes and Offices at the NIH, the NIDDK is also supporting an initiative, "Environmental Approaches to the Prevention of Obesity," a research solicitation that is establishing studies of preventive approaches targeting environmental factors that contribute to inappropriate weight gain in children, adolescents, and adults—another critical aspect of obesity.

The NIDDK's public education efforts related to obesity include the Weight-control Information Network, and the National Diabetes Education Program (see sidebars here and in the "Diabetes, Endocrinology, and Metabolic Diseases" chapter for further descriptions of both of these efforts). The latter is a partnership among the Centers for Disease Control and Prevention, the NIDDK, and approximately 200 public and private organizations.

Finally, the NIDDK supports all of these programs with a solid base of fundamental research on biologic processes such as nutrient metabolism and how it is influenced by genetic and environmental factors.

Air EL, Strowski MZ, Benoit SC, Conarello SL, Salituro GM, Guan X-M, Liu K, Woods SC and Zhang BB: Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. *Nat Med* 8: 179-83, 2002.

Bachman ES, Dhillon H, Zhang C-Y, Cinti S, Bianco AC, Kobilka BK and Lowell BB: BetaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297: 843-5, 2002.

Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM and Friedman JM: Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 297: 240-3, 2002.

Cohen P, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P and Friedman JM: Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 108: 1113-21, 2001.

Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD and Elmquist JK: Activation of central melanocortin pathways by fenfluramine. *Science* 297: 609-11, 2002.

Kumar MV, Shimokawa T, Nagy TR and Lane MD: Differential effects of a centrally acting fatty acid synthase inhibitor in lean and obese mice. *Proc Natl Acad Sci USA* 99: 1921-5, 2002.

Stone S, Abkevich V, Hunt SC, Gutin A, Russell DL, Neff CD, Riley R, Frech GC, Hensel CH, Jammulapati S, Potter J, Sexton D, Tran T, Gibbs D, Iliev D, Gress R, Bloomquist B, Amatruda J, Rae PMM, Adams TD, Skolnick MH, and Shattuck D: A major predisposition locus for severe obesity, at 4p15-p14. *Am J Hum Genet* 70: 1459-68, 2002.

T CELLS AND LIVER DAMAGE IN PRIMARY BILIARY CIRRHOSIS

The liver, the largest organ in the body, is essential for keeping the body functioning properly. It removes or neutralizes poisons from the blood, produces immune agents to control infection, and removes germs and bacteria from the blood. It makes proteins that regulate blood clotting and produces bile to help absorb fats and fat-soluble vitamins. Liver diseases that interfere with these essential functions can therefore severely threaten health (see "Patient Profile: Chris Klug").

Like type 1 diabetes (see "Diabetes, Endocrinology, and Metabolic Diseases" chapter), primary biliary cirrhosis is an autoimmune disease—in this case, one that destroys liver cells. Immune system cells normally protect the body from invaders such as bacteria and viruses, but in the case of primary biliary cirrhosis and other "autoimmune" diseases, some misguided immune system cells attack one of the body's own proteins or cell types. Researchers had previously implicated several different types of immune system cells as having roles in primary biliary cirrhosis and had also identified the target of these autoimmune attacks: a protein called PDC-E2. In experiments designed to elucidate the molecular mechanisms underlying primary biliary cirrhosis, NIDDK-supported scientists have now found evidence that particularly destructive types of immune system cells, called CD8+ T cells, may contribute to the extensive liver damage associated with this disease.

With an advanced technique for detecting particular types of T cells, the NIDDK-supported researchers recently found that CD8+ T cells that recognize the PDC-E2 protein—and that will kill cells that carry PDC-E2—are enriched in the livers of patients with primary biliary cirrhosis. When the scientists compared blood samples with liver tissue from patients, they found that the frequency of these destructive, PDC-E2-reactive T cells in the liver was 10 times higher than that in the blood. This finding was consistent with the greatly enriched number of precursors to CD8+ PDC-E2 reactive T cells that the researchers identified in primary biliary cirrhosis patients as compared to other chronic liver disease patients and normal "control" patients.

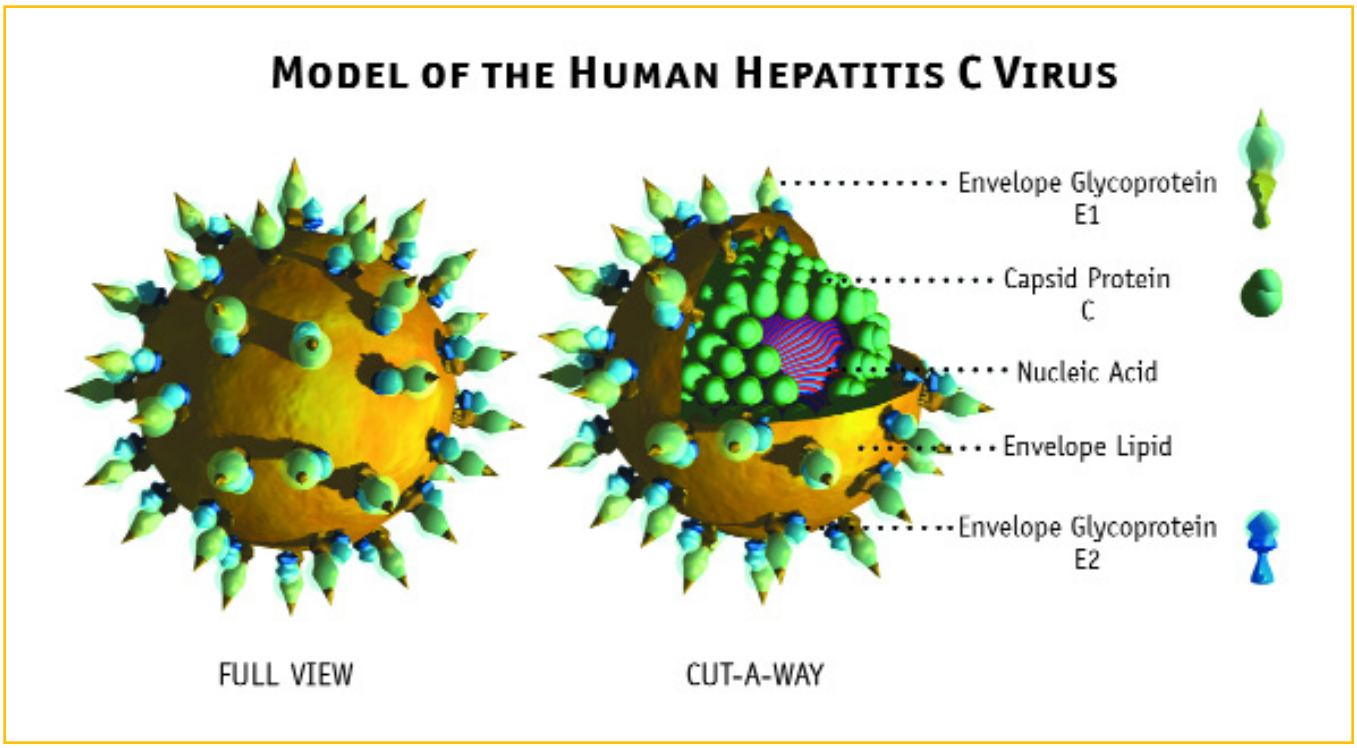
These data not only suggest that CD8+T cells are involved in the pathology of primary biliary cirrhosis, but also confirm the importance of the PDC-E2 protein as a central target for the autoimmune attack in this disease. By building upon our knowledge of the types of immune cells involved in liver damage in primary biliary cirrhosis and the target(s) involved, researchers are gaining insights that may ultimately lead to new therapies for this destructive liver disease.

Kita H, Matsumura S, He XS, Ansari AA, Lian ZX, Van de Water J, Coppel RL, Kaplan MM, and Gershwin ME: Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. *J Clin Invest* 109: 1231-40, 2002.

NEW INSIGHTS FROM BASIC AND CLINICAL RESEARCH ON HEPATITIS C

Hepatitis C virus is one of the most common causes of liver disease in the U.S. It accounts for about 15 percent of acute viral hepatitis, 60 to 70 percent of chronic hepatitis, and up to 50 percent of cirrhosis, end-stage liver disease, and liver cancer. An estimated 4 million Americans have antibody to HCV (anti-HCV), indicating ongoing or previous infection with the virus. Hepatitis C causes an estimated 8,000 to 10,000 deaths annually in the U.S. alone.

While current drug treatments eliminate the virus in many people, they are totally ineffective or minimally effective in others, and it has not been clear whether a vaccine would be a useful alternative means for protecting against hepatitis C. Additionally, despite its proficiency at human infection, hepatitis C virus has been generally refractory to scientists' efforts to induce infections in small laboratory animals for use as models for



A three-dimensional model of the human hepatitis C virus. Researchers estimate that at least 20 percent of patients with chronic hepatitis C develop cirrhosis, a process that takes 10 to 20 years. After 20 to 40 years, a smaller percentage of patients with chronic disease develop liver cancer. Illustration credit: Three-dimensional model of HCV created by Louis E. Henderson, Ph.D. is reproduced with permission from The PRN Notebook,™ Vol. 6, No. 1, March 2001. Published by Physicians' Research Network, Inc.,® New York, NY, USA. All rights reserved. ©March 2001. For further information visit www.prn.org.

research. One group of NIDDK-supported scientists has now developed a novel mouse model of hepatitis C disease, while another NIDDK-supported research team has investigated whether protection against viral persistence might be possible in humans.

When hepatitis C virus infects humans, it inserts its genetic material into cells, so that the cells will produce viral proteins. Because the virus does not normally infect mice, a group of scientists came up with another way to put viral genes into these animals: they injected the genes into mouse eggs to generate transgenic mice that carried either the entire viral genome or a subset of the genes. The scientists had also linked the viral genes together with a segment of mouse regulatory DNA that turned on the genes in the liver. The genetically engineered mice accumulated excess fat in their livers and developed liver tumors, conditions commonly seen in hepatitis C infections in people. At the same time, because the viral genes were innate to the transgenic mice and not introduced by infection, the potentially injurious inflammation that accompanies infection in humans was absent in these mice. The scientists concluded that viral proteins influence hepatitis C symptoms, although inflammation may also contribute to disease symptoms in human infection.

Vaccines for viral diseases are essentially mock infections with a weakened virus or virus fragment; they train the body to fight off later infections with a real virus. To see whether a vaccine approach might be useful for hepatitis C, scientists recently studied a group of people likely to have multiple exposures to this virus: users of injectable drugs. Some drug users had evidence of an earlier infection from which they had since recovered, while others had not been infected previously. Observations over

the course of two years showed that those who had previous infections were over 10 times less likely to acquire a new persistent infection than those who had not been previously infected. In many of the people, prior infection seemed to confer protection against subsequent, persistent infections. Therefore, the scientists concluded that a vaccine approach to hepatitis C could be beneficial, because the serious liver diseases caused by this virus are associated with its persistence in the liver. This study also revealed that users of injectable drugs have an alarmingly high incidence of hepatitis C virus infection.

These studies provide insights into how hepatitis C wreaks havoc in the liver and how some level of protection against persistence of this virus in the body, and associated disease, might be achieved. This research will likely spur new efforts toward vaccine development.

Providing further support for these research efforts, the NIDDK was one of the primary sponsors of a recent NIH consensus conference on management of hepatitis C (see sidebar, "Hepatitis C Consensus Conference"), to review the current state of knowledge about the disease and treatment options and to identify the most pressing questions for basic and clinical researchers to tackle in the near future.

The NIDDK is also currently supporting two major clinical trials addressing hepatitis C infection. The Hepatitis C Antirviral Long-term Treatment against Cirrhosis (HALT-C) trial is designed to investigate whether long-term treatment of hepatitis C with the drug peginterferon-alfa will prevent progression of liver disease in patients for whom prior treatment did not eliminate the virus. The Virahep-C study will examine resistance to antiviral therapy in patients with chronic hepatitis C, specifically focusing on African Americans, among whom such viral resistance is common. Enrollment for this study has just begun.

Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, Okuda M, Gosert R, Xiao S-Y, Weinman SA, and Lemon SM: Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. *Gastroenterology* 122: 352-65, 2002.

Mehta SH, Cox A, Hoover DR, Wang XH, Mao Q, Ray S, Strathdee SA, Vlahov D, and Thomas DL: Protection against persistence of hepatitis C. *Lancet* 359: 1478-83, 2002.

INFLAMMATORY BOWEL DISEASE

The inflammatory bowel diseases (IBD) known as Crohn's disease and ulcerative colitis affect nearly one million Americans. Typical early symptoms of IBD include abdominal pain, fever, watery or bloody diarrhea and weight loss, which can progress to malnutrition and growth retardation, compromise employment and social activities, and increase risk of intestinal cancer (see "Patient Profile: Rachel Hettich—Living with Crohn's Disease"). Although the symptoms are similar, a distinguishing characteristic between Crohn's disease and ulcerative colitis is the location of the inflammation associated with them. The precise cause or causes of IBD are not yet known, but researchers are accumulating new insights into these diseases.

Susceptibility Genes in IBD: One of the theories about the development of IBD is that the patient's immune system reacts inappropriately to bacteria that normally reside in the gut. This reaction initiates a cascade of molecular events that results in inflammation in the gastrointestinal tract. IL-10 is an anti-inflammatory molecule naturally produced in the body that, along with many other molecules, regulates inflammation in IBD. Molecules such as IL-10 that might provide resistance and others that might increase the disease inflammation are the products of individual genes that can be considered "susceptibility" genes. Because of their often small though significant contributions to a disease process, susceptibility genes can be difficult to identify, especially in complex diseases such as IBD. Nonetheless, finding these genes is very important for understanding how a disease develops.

To search for genes that influence IBD severity, NIDDK-supported researchers used a state-of-the-art technique known as quantitative trait locus (QTL) mapping in mice to identify chromosomal regions (loci) in which such genes may be located. For the study, the researchers first selected two strains of IL-10-deficient mice that develop disease that has some similarities to human IBD. One strain of mice is highly susceptible to gut inflammation, while the other is relatively resistant. The scientists then cross-mated mice from each strain and analyzed their hybrid descendants, which carried chromosomal DNA from both original strains.

By quantitating the severity of disease symptoms in the mice and correlating the symptoms with the inheritance of different chromosomal loci, the scientists identified loci that are linked to IBD severity in IL-10-deficient mice. The most significant of these is on chromosome 3. The version of this locus that was inherited from the highly IBD-susceptible strain exacerbated disease, affecting nearly all of the symptoms that were studied. Loci on other chromosomes, including, interestingly, versions of loci from the relatively IBD-resistant strain, also contributed to disease symptoms. Further experiments, in which various combinations of these loci were analyzed, showed that the genetic complexity of IBD arises not only from the multiple loci that control its severity, but also from the different types of effects caused by genetic interactions among these loci.

The complete sequence of the mouse genome is now being assembled, providing sequence information about genes in mice that are homologous to human genes; if the mouse genes on chromosome 3 that contribute to IBD severity can be identified, then it may be possible to identify similar genes which cause disease susceptibility in humans. This study emphasizes the complexity of molecular events leading to IL-10 deficiency-induced IBD in mice. The results provide new knowledge about the genetic underpinnings of IBD, but also emphasize the difficulties to be overcome in finding therapies for patients.

Improving Diagnostics for IBD Lesions: Until now it was not easy to delineate between the two types of colorectal pre-cancerous lesions, ordinary sporadic colorectal adenomas and cancers (SAC) and a type of colon neoplasm associated with IBD, flat inflammatory bowel disease dysplasia (IBDN). The treatments of choice for these two types of lesions are very different when they can be identified. SACs may be removed by a colonoscopy or a surgical procedure, whereas IBDNs may require removal of the entire colon.

In a recent study, NIDDK-supported researchers employed a sophisticated technology known as artificial neural networks (ANN) to distinguish between the two types of lesions. ANNs are mathematical computer models designed to mimic the mammalian brain. They are composed of a large number of processing elements known as "neurons" that are connected by "synapses" that store information. ANNs are unique in their ability to "learn" complex patterns by example. Once information is stored in the synapses, ANNs can apply it to analyze unknowns through pattern recognition.

For this study, researchers used samples of damaged tissue from patients who were diagnosed as having either SAC or IBDN. Gene expression profiling of the tissue generated the data needed to "train" the ANN. After these data were processed and stored in "synapses," the ANN was presented with information derived from 12 new SAC or IBDN patients. The ANN was able to correctly identify all 12 samples as being either SAC or IBDN. This novel system of analyzing data has given clinicians the tools needed to accurately classify SACs and IBDNs and to choose the optimal treatment for their patients. This will prevent SAC patients from unnecessary colonectomies and will ensure that patients with IBDN receive the treatment they require.

Farmer MA, Sundberg JP, Bristol IJ, Churchill GA, Li R, Elson CO, and Leiter EH: A major quantitative trait locus on chromosome 3 controls colitis severity in IL-10-deficient mice. *Proc Natl Acad Sci USA* 98: 13820-5, 2001.

Selaru FM, Xu Y, Yin J, Zou T, Liu TC, Mori Y, Abraham JM, Sato F, Wang S, Twigg C, Olaru A, Shustova V, Leytin A, Hytiroglou P, Shibata D, Harpaz N and Meltzer SJ: Artificial neural networks distinguish among subtypes of neoplastic colorectal lesions. *Gastroenterology* 122: 606-13, 2002.

POINTING THE WAY TO ACUTE PANCREATITIS THERAPIES

One of the many functions of the pancreas is to produce the enzymes necessary for digestion. Normally, digestive enzymes are synthesized in the pancreas in an inactive form called a zymogen. They then travel to the small intestine where they are activated and aid digestion by breaking down the fats, proteins, and carbohydrates in food.

Because activated digestive enzymes would start digesting the pancreas if given the opportunity, there are molecular safeguards in place to keep enzyme activation from occurring prematurely. The primary safeguard is that zymogens become activated when an inhibitory piece of protein is clipped off, and this clipping can normally only occur in the chemical conditions present in the small intestine. However, the safeguards sometimes break down, and disease results (see also "Story of Discovery: Genetic Insights into Pancreatitis and Pancreatic Cancer").

Acute pancreatitis is a complex disease that causes inflammation and destruction of pancreatic tissue. In the U.S. there are approximately 80,000 individuals affected each year. In acute pancreatitis, enzymes are activated prematurely in the pancreas and begin to "digest" it. If sufficient damage occurs, active enzymes are released into the bloodstream and transported to other major organs of the body, where they continue their destruction.

The digestive enzyme trypsin is known to be a key player in the events leading to pancreatitis. A recent study has determined that an enzyme called PI3K plays a role in the activation of trypsin. Researchers showed that premature trypsin activation can be reduced, and the severity of acute pancreatitis ameliorated, by inhibitors of PI3K. From this research, it is now clear that PI3K plays a crucial role in trypsin activation and the onset of acute pancreatitis. These studies point the way to the development of potential therapies that can halt this step in the cascade of events responsible for acute pancreatitis.

Singh VP, Saluja AK, Bhagat L, van Acker GJD, Song AM, Soltoff SP, Cantley LC and Steer ML: Phosphatidylinositol 3-kinase-dependent activation of trypsinogen modulates the severity of acute pancreatitis. *J Clin Invest* 108: 1387-95, 2001.

A MOLECULAR PROFILE OF MOUSE STOMACH CELLS BEFORE AND AFTER *H. PYLORI* INFECTION

While the stomach has long been known for its role in food digestion, until recently the genes that are active in stomach cells have been elusive. This past year, scientists began a molecular characterization of the different kinds of cells in the stomach to learn which genes are important for stomach function. They also sought to discover genes that are turned on in response to infection with *Helicobacter pylori,* a bacterium linked to ulcers and stomach cancer. The scientists began with the parietal cell, a highly specialized acid-secreting cell of the stomach.

For cataloguing genes expressed (turned on) in the stomach, the choice of the parietal cell arose in part from biomedical considerations: scientists knew that parietal cells secrete acid, and diseases associated with stomach acid are common. Previous studies hinted that these cells may perform other important functions as well. There were also practical considerations: in order to survey genes that are specifically activated in a given type of cell, the researchers needed to be able to separate that cell type from other stomach cells. They were able to do just this for parietal cells by mixing mouse stomach tissue with special magnetic beads. These beads had been coated with a substance that sticks to particular molecules on the surface of parietal cells. The scientists then pulled the magnetic beads—and the attached parietal cells—out of the mixture with a magnet to separate them from the other stomach cells.

Using microarray technology, the researchers then scanned nearly 11,000 mouse genes and identified a set of genes expressed in parietal cells, but not in the other stomach cells. This set not only included genes known to be associated with parietal cell activities, such as acid secretion, but also revealed genes that may play roles in previously unappreciated aspects of parietal cell function, including the regulation of stem cell proliferation in the stomach. To see how parietal cells and other stomach cells react to *H. pylori* infection, the scientists compared the profile of expressed genes in stomach cells of mice before and after infection. While gene expression in the parietal cells remained stable, the other stomach cells commenced a flurry of genetic activity in response to the *H. pylori* infection. The cells turned on genes involved in immune system signaling, genes that help respond to the presence of bacterial molecules, genes that aid in the repair of damaged tissue, and many other genes.

This functional genomics approach to the study of the stomach provides important information not only about normal stomach cell functions, but also about how stomach cells respond to infection. The scientists found a set of genes that were consistently enriched in parietal cells under a variety of conditions. This database of genes provides a molecular signature of parietal cells that will serve as a resource for studying these cells in different disease states and for evaluating how the cells react to different medicines. By identifying the non-parietal cells of the stomach as key responders to *H. pylori* in mice, and by illuminating the repertoire of genes activated in response to this bacterium, the scientists have also opened new avenues of research into this potentially serious infection.

Mills JC, Syder AJ, Hong CV, Guruge JL, Raaii F, and Gordon JI: A molecular profile of the mouse gastric parietal cell with and without exposure to *Helicobacter pylori*. *Proc Natl Acad Sci USA* 98: 13687-92, 2001.

ONGOING AND NEWLY LAUNCHED NIDDK EFFORTS IN DIGESTIVE DISEASES AND NUTRITION

The NIDDK is currently addressing numerous basic and clinical research challenges and opportunities in order to advance knowledge and therapies for digestive diseases. For example, the NIDDK is intensifying its efforts to combat obesity as a serious health problem and as a risk factor for type 2 diabetes. The Look AHEAD (Action for Health in Diabetes) multi-center clinical trial is under way and has reached approximately the midpoint of its recruitment goal of 5,000 individuals. This long-term clinical trial is designed to answer two major questions. First, do interventions designed to produce voluntary sustained weight loss in obese people with type 2 diabetes improve health, particularly with respect to cardiovascular outcomes? Second, how do these interventions compare with treating obesity-related conditions without weight loss? Results of this study will help guide future patient care.

As noted previously, the NIDDK and other NIH Institutes are co-sponsoring an initiative, "Environmental Approaches to the Prevention of Obesity." Through this research solicitation, the NIH is establishing studies of preventive approaches targeting environmental factors that contribute to inappropriate weight gain in children, adolescents, and adults. Another facet of research on obesity will be to promote clinical research on bariatric surgery, currently used in treating extreme obesity, to better understand the impact of bariatric surgical procedures on obesity and related co-morbid conditions. At the same time, a strong foundation of basic and pre-clinical research is critical for understanding and developing new preventive or interventional therapies for obesity. The continued elucidation of the molecular factors and pathways responsible for appetite regulation, metabolism, and energy storage offers rich prospects for the development of new drugs that will promote safe and effective long-term weight loss.

Through a recent initiative, the NIDDK hopes to support more research to provide new insights into intestinal failure, short gut syndrome, and small bowel transplantation. To strengthen research in inflammatory bowel disease (IBD), the NIDDK will accelerate efforts to identify additional genes or genomic regions associated with increased risk of IBD or with clinical manifestations of IBD through a newly established IBD Genetics Research Consortium.

Research will also be bolstered on hepatitis C virology, epidemiology, natural history, prevention, and therapy—potentially including research on therapy in children. Recently, the Institute launched a new effort to elucidate the clinical features and pathogenesis of drug- and toxin-induced liver injury, common causes of acute liver disease, morbidity, and mortality. As already noted, the NIDDK is continuing enrollment of patients in the multi-center Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) clinical trial. This trial is designed to investigate whether long-term treatment of hepatitis C with peginterferon-alfa will prevent progression of liver disease in patients for whom prior treatment did not eliminate the virus. Enrollment has also begun for the Virahep-C study, which will examine resistance to antiviral therapy in patients with chronic hepatitis C, specifically focusing on African Americans, among whom such viral resistance is common. New clinical research efforts are also now beginning on two other liver diseases, non-alcoholic steatohepatitis and biliary atresia.

Finally, the NIDDK has launched a significant new effort in liver transplantation. Liver transplantation is the only cure for people with end-stage liver disease. Yet, over 17,000 Americans are awaiting transplantation due to the shortage of cadaveric livers available for transplant. The NIDDK, in collaboration with the Federal Health Resources and Service Administration (HRSA) and the American Society of Transplant Surgeons, recently launched the Adult-to-Adult Living Donor Liver Transplant Cohort Study (A2ALL) to carefully evaluate the risks and patient outcomes for donors and patients undergoing this procedure. This procedure enables a patient to receive part of a liver from a living donor, rather than a cadaver; because of the liver's amazing ability to regenerate, the donor's liver eventually regrows to full size, and the transplanted portion also grows in the recipient. The procedure is gaining widespread use, but risks to potential donors need to be comprehensively assessed and uniform criteria for matching donors and recipients are needed. The A2ALL study should provide valuable information to both patients and physicians.

VISION STATEMENT

Rena Wing, Ph.D.

The Diabetes Prevention Program: A Successful Approach to Lifestyle Intervention

Dr. Rena Wing is Professor of Psychiatry and Human Behavior at Brown Medical School—The Miriam Hospital, in Providence, Rhode Island. She was the coordinator of the intensive lifestyle intervention arm of the recently completed Diabetes Prevention Program clinical trial, sponsored by the NIDDK. Dr. Wing's research interests include the development of behavioral treatments for obesity, particularly as it relates to type 2 diabetes. Her research team currently is participating in the Look AHEAD (Action for Health in Diabetes) project, the first major nationwide study to look at the long-term health effects of weight loss in men and women who are overweight and have type 2 diabetes. Dr. Wing is the Chair of the LookAHEAD study. She also is participating in the follow-up study to the Diabetes Prevention Program, the Diabetes Prevention Program Outcomes Study. Both of these studies are sponsored by the NIDDK.

At the May 2002 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Wing presented an overview of the intensive lifestyle intervention arm of the Diabetes Prevention Program (DPP) clinical trial. This aspect of the study yielded remarkable findings about how diet combined with physical activity can reduce the risk of developing type 2 diabetes in individuals who are at high risk for the disease. Trial participants lost an average of 5-to-7 percent of their body weight and performed at least 150 minutes of physical activity per week. These changes reduced their risk of developing diabetes by 58 percent over an average 2.8 year follow-up period. Rigorous, systematic, and controlled testing of the lifestyle hypothesis through the DPP provided definitive proof that prevention of type 2 diabetes is possible through positive lifestyle changes. Dr. Wing emphasized that perhaps the most important factor contributing to the success of the intensive lifestyle intervention was a history of several decades of investment in programmatic basic behavioral intervention research. It was this strong research foundation that enabled DPP investigators to design an effective weight loss program.

Dr. Rena Wing

According to Dr. Wing, the DPP is an example of an extremely effective behavioral intervention. This intervention was based on prior observational studies suggesting that modest changes in weight or physical activity might reduce the risk of developing type 2 diabetes. Clinical trial data from other studies also suggested that modest changes in weight and physical activity could be produced.

Biobehavioral Arm of the DPP

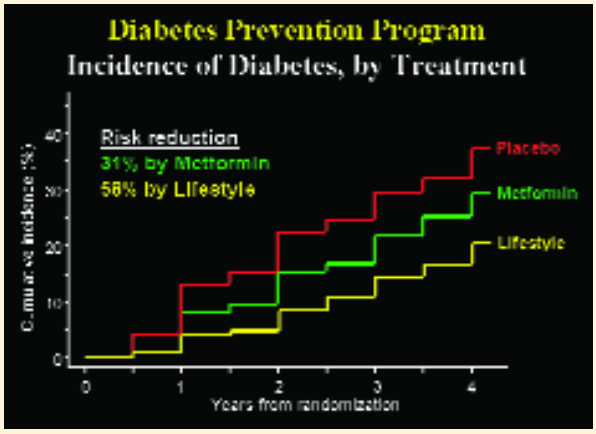
Based on previous behavioral studies, to achieve the weight loss goal, DPP participants were instructed to reduce their dietary fat intake to less than 25 percent of total calories and they were given a calorie intake goal of 1,200 to 2,000 calories daily depending on their initial body weight.

Previous studies also showed that the combination of diet and exercise was particularly effective for long-term maintenance of weight loss. Therefore, the DPP investigators viewed physical activity as important for achieving and maintaining weight loss. The strategy for achieving the physical activity goal was to stress brisk walking and other activities of similar intensity, such as aerobic dance or bicycling. Prior studies also suggested that even short bouts of exercise—as little as 10 minutes—could be very helpful in promoting weight loss and improving fitness. This concept was employed in the DPP, in which participants were encouraged to exercise at least 3 days per week, with at least 10 minutes per session, for a total of at least 150 minutes per week. All of the DPP clinical centers were required to offer two supervised exercise sessions per week to help participants achieve the activity goal.

A key feature of the lifestyle component of the DPP was the use of frequent contact and ongoing intervention. Research has shown that positive reinforcers, contingency contracts, and, in particular, frequent and intensive contact, can help individuals maintain their behavior changes. Thus, in the DPP, all participants were assigned a case manager who worked with them in a one-to-one manner on a weekly to monthly basis throughout the trial. In addition, DPP centers offered three group courses per year during the maintenance phase, usually with courses offered on diet, exercise, and behavior. Three to four motivational campaigns were conducted each year in which participants competed among others in their center or across DPP study centers to see who could achieve the weight loss or exercise goals.

Applicability of DPP Results

In addition to reducing the development of diabetes by 58 percent overall, the DPP intervention yielded consistent results across diverse subgroups. Both men and women and all ethnic/racial groups benefited from the lifestyle intervention. It is important to realize that there are an estimated 16 million Americans with "pre-diabetes" who resemble participants in the DPP with respect to their risk



Cumulative incidence of diabetes according to the Diabetes Prevention Program (DPP) study group.

for developing type 2 diabetes. If they adopt lifestyle changes similar to those in the DPP, their disease risk might be reduced by over 50 percent.

Surprisingly, the lifestyle intervention worked best in older individuals, those over age 60. Contrary to the DPP investigators' concern that many participants over 60 might not be compliant with the lifestyle intervention, this age group actually demonstrated the best adherence to the weight loss and physical activity intervention goals. This result is significant because type 2 diabetes is considerably more prevalent in the over 60 age group.

General Implementation of Lifestyle Intervention

With completion of the DPP, Dr. Wing is turning to the future and to the general implementation of the intensive lifestyle intervention to prevent type 2 diabetes. How is the DPP helping shape the future behavioral research agenda? Dr. Wing notes that the DPP suggests certain areas of particular importance for future research. The one she believes is the highest priority is research on how we can help people maintain their behavior change. "With the most intensive, best program we could develop, our lifestyle participants achieved their best weight losses at 6 months, maintained them through a year, and then gradually regained," she says. "They regained even though we were giving them intensive contact, and were trying everything we could do. Yet, they still

regained. This is the problem we have in the field. I think this is the number one priority for our research, that is, to understand how we can help individuals maintain various types of behavior change and thereby maintain their weight loss long-term."

Dr. Wing believes there are three approaches to the maintenance of behavior change on which research should be encouraged. One is to study people who have successfully changed their behavior long-term. "We need to understand how they do it—how they are able to succeed," she says. A second approach is to understand more fully why maintenance is so difficult. "What happens at six months or a year that makes people start to regain? Is it a physiological change or is it a behavioral problem that leads them to regain?" Lastly, Dr. Wing believes there is a need to encourage investigators to develop innovative strategies for long-term maintenance of behavior change. She notes that: "What we're doing isn't working as well as it should be, and I think we need to encourage creativity and innovative approaches."

Some of Dr. Wing's own research on the maintenance of weight loss indicates that low-calorie diets and, more importantly, low-fat diets are critical to maintaining weight loss long-term. Also important is a relatively high level of physical activity—up to 2,800 calories per week, the equivalent of walking four miles a day, 7 days a week. Dr. Wing stresses, however, that new and innovative approaches to improving long-term maintenance of weight loss must be developed.

Dr. Wing also believes that, to apply the results of the DPP to the general population, it is important to disseminate the DPP message effectively. She is looking at the Internet as one means of disseminating treatments to a large number of people, observing that, "it's increasingly popular, it has no geographic limitations, is convenient to people, and is interactive between a therapist and a participant. There are also opportunities for support among participants." Offering both educational materials and a structured behavioral program on the Internet, Dr. Wing and her colleague Dr. Tate recently reported that, at both the three-month and the six-month point, the Internet behavior therapy program was far more effective than the Internet educational program in changing participants' body weight. The researchers were able to produce a weight loss of about nine pounds through the Internet behavioral program. They are now working to develop an even more effective behavioral program that includes initial weight loss and maintenance of weight loss. "Again, we need to be applying behavioral principles," Dr. Wing says, "carefully looking at how to change the cues and the consequences in the environment so as to maintain the behavior change. I think we really need to be studying how to intervene on the whole environment, particularly the home, where most meals are eaten."

On the impact of the DPP, Dr. Wing says, "I think we have shown that we can conduct studies looking at how behavior change affects health outcomes. I think the DPP has opened the door for further study of the impact of participation in weight loss programs on other health outcomes. I'm thrilled that the NIH has moved to initiate the Look AHEAD study. This is a study examining the long-term health impact of participation in weight loss intervention on cardiovascular morbidity and mortality in 5,000 obese individuals with type 2 diabetes. The study will also be looking at many other health consequences of weight loss, including diabetes complications, changes in cardiovascular risk factors, hospitalizations, and the cost and cost-effectiveness of this lifestyle intervention."

Future Directions in Behavioral Research

Dr. Wing has identified several major topics for future behavioral research as it relates to improving health. These include small-scale basic behavioral studies focused on improving maintenance of behavior change, disseminating the discovery of new treatments, and interventions to change the environment and thereby change behavior. "With knowledge gained from these studies," she says, "investigations can be conducted to examine how lifestyle change affects health outcomes."

WIN: The Weight-Control Information Network

When the Department of Health and Human Services (HHS) released its report entitled, *The Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity,* HHS Secretary Tommy G. Thompson warned that, "Overweight and obesity are among the most pressing new health challenges we face today." It has been estimated that 300,000 deaths each year in the U.S. are associated with obesity and overweight and the numbers are increasing. Obesity is an epidemic that must be brought under control. The WIN is an NIDDK service directed at reaching this goal.

Established in 1994, the NIDDK's Weight-control Information Network (WIN) is a national information service that produces and provides science-based information on obesity, physical activity, weight control, and nutrition to health professionals, people who are overweight or obese, consumers, the media, and Congress. The WIN has reached out to all age groups and diverse ethnic and racial groups with its materials.

The WIN is publishing a new series of booklets, *Healthy Eating and Physical Activity Across Your Lifespan,* to encourage better eating and physical activity habits. The series contains four booklets entitled, *Tips for Parents, Tips for Adults, Tips for Older Adults,* and the upcoming, *Tips for Pregnant Women*. These booklets are published in both English and Spanish language versions.

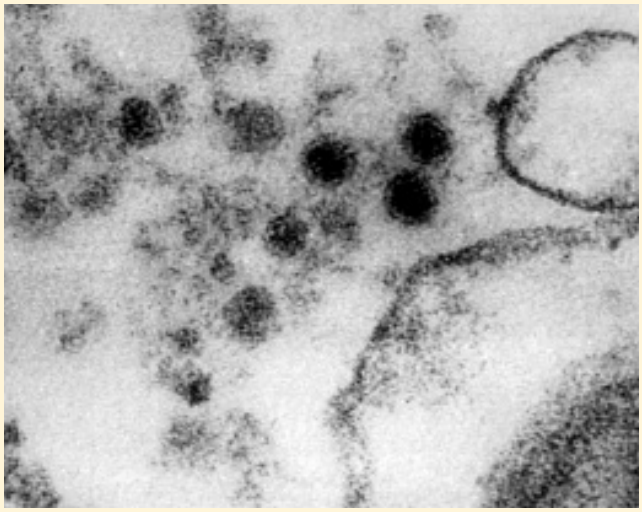
The WIN's "Sisters Together: Move More, Eat Better" initiative encourages African-American women 18 and older, who are disproportionately affected by overweight and obesity, to maintain a healthy weight by increasing physical activity and eating healthier food. A pilot program of "Sisters Together" and its materials was held in Boston in the mid-late 1990's. A planning guide and kit based on the "Sisters Together: Move More, Eat Better" pilot program are available to provide step-by-step instructions for planning, promoting, implementing and evaluating community health awareness programs to prevent African-American women from becoming overweight. "Sisters Together" has also produced other informational brochures.

The WIN is also coordinating with the Institute's Look AHEAD (Action for Health in Diabetes) clinical trial. The Look AHEAD trial is a large scale (anticipated 5,000 participants) multi-center trial that will examine whether a lifestyle intervention designed to achieve voluntary long-term weight loss will improve cardiovascular and other outcomes in obese individuals with type 2 diabetes. The WIN will provide information on physical activity and healthy eating to trial participants.

Hepatitis C Consensus Conference

The NIH's Consensus Development Program brings together scientific experts to consider current evidence and produce consensus statements addressing controversial issues in medicine. NIH Consensus Statements are disseminated widely to healthcare practitioners, healthcare policymakers, patients, the general public, and the media, and serve as a valuable resource to these groups when they make decisions that impact health.

The NIH convened its original Consensus Development Conference on Management of Hepatitis C in March 1997. Because knowledge of hepatitis C has dramatically increased in the past 5 years, a June 2002 conference was convened to revise and reissue the original 1997 Consensus Panel statement. The NIDDK and the NIH's Office of Medical Application of Research (OMAR) were the primary sponsors.



An electron micrograph of Hepatitis C virus particles. Infection with the hepatitis C virus is the most common reason for liver transplantation in the U.S. Photo: Dr. Jake Liang, NIDDK.

The hepatitis C virus (HCV) is one of the most important known causes of chronic liver disease in the U.S. It is a common cause of cirrhosis and hepatocellular carcinoma (HCC, liver cancer), and it is the most common reason for liver transplantation. An estimated 4 million Americans have antibodies to HCV, indicating ongoing or previous infection with the virus. Chronic HCV infection can produce a wide spectrum of outcomes—from little or no liver damage to development of cirrhosis and end-stage liver disease. This wide variability in outcomes makes it particularly challenging for healthcare practitioners deciding how best to diagnose and treat the disease. In such instances, consulting a consensus statement may help them to make informed decisions.

During the first portion of the conference, experts presented the latest hepatitis C research findings to an independent, non-Federal Consensus Development Panel. The panel also considered systematic literature reviews and analyses of controversial hepatitis C topics, presented by the Johns Hopkins University Evidence-Based Practice Center. These included: (1) the role of screening for hepatocellular carcinoma, (2) the role of liver biopsy, and (3) the optimal therapeutic regimens for hepatitis C. After carefully weighing the scientific evidence, the panel drafted a consensus statement targeted at practicing physicians, health care workers, and the general public. On the final day of the conference, the panel chairperson read the draft statement to the audience and invited questions and comments.

The statement provides detailed information and/or recommendations on six major questions:

1. What is the natural history of hepatitis C?
In this section, the authors consider known features of the hepatitis C virus (HCV), the epidemiology of hepatitis C infection, characteristics of both acute and chronic infection, and extrahepatic (or outside the liver) manifestations of hepatitis C infection.

2. What is the most appropriate approach to diagnose and monitor patients?
Here, the authors discuss a variety of possible tests to detect or imply hepatitis C infection, including tests that detect antibodies to HCV, HCV RNA assays, testing for liver enzyme levels suggestive of HCV infection, noninvasive liver scarring tests, liver biopsy, and screening for two diseases commonly associated with HCV, namely hepatocellular carcinoma and HIV/AIDS.

3. What is the most effective therapy for hepatitis C?
The authors discuss improvements in therapy since the original 1997 consensus conference on hepatitis C, including combination therapy with ribavirin and the introduction of pegylated interferons. They describe pros and cons of using these therapies in different patient populations as well as characteristics of patients and of different hepatitis C viral genotypes that influence treatment success. Information on re-treatment of patients, adherence to therapy regimens, and side effects is also included.

4. Which patients with hepatitis C should be treated?
Because of the high cost, variable risk of developing serious liver damage, and complications possible with antiviral therapy, not all HCV-infected patients are recommended for treatment. The authors discuss treatment options for differing liver disease severity (as indicated by liver enzyme levels), recurrence after liver transplantation, HCV-infected children, acute HCV infection, active injection drug users, alcohol abusers, and HIV co-infected individuals.

5. What recommendations can be made to patients to prevent transmission of hepatitis C?
Because injection drug use accounts for two-thirds of all new HCV infections in the U.S., the authors discuss options for reversing this trend. Other risk factors for infection that may be controllable include sexual intercourse with an infected individual, exposure to infected blood or blood products, needlestick injuries, body piercing and tattooing, and transmission to a baby from an infected mother. Suggestions for preventing transmission are made for each situation.

6. What are the most important areas for future research?
The authors identify an extensive list of important research areas, including areas of basic science, clinical research, clinical trials, educational programs, infrastructural support, and statistical research.

The Consensus Development Panel's draft statement was posted online on Wednesday, June 12, 2002. The final statement is now available at this URL: http://consensus.nih.gov/cons/116/ 116cdc_intro.htm. The NIH Consensus Development Program's website is located at: http://consensus.nih.gov/.

This statement reflects the panel's assessment of medical knowledge on management of hepatitis C as of June 2002. Even as the statement is read and used, newer knowledge is constantly accumulating through medical research.

PATIENT PROFILE

Rachel Hettich

Living with Crohn's Disease

Most teenage girls are concerned about gaining weight. Rachel Hettich wishes she had that privilege. At age eight, after undergoing a battery of tests—including a variety of blood tests and a colonoscopy—she was diagnosed with a severe case of Crohn's disease. Crohn's disease is an inflammatory bowel disease (IBD) that causes inflammation in the gastrointestinal tract (it can involve any site from the mouth to the anus).
"In third grade I began losing lots of weight and experiencing excruciating stomach pains that wouldn't go away," says 18-year-old Rachel, a high school senior who, because of Crohn's disease, misses more days of school than she attends.
At the time of her diagnosis, neither Rachel nor her family had any knowledge or understanding of the disease, or of the devastating impact it would exert on all of them. Unfortunately, it wouldn't take long for them to learn.

In Crohn's disease, the immune system attacks cells in the intestines. This causes severe abdominal pain, fever, ulcers and other conditions that can become disabling, including diarrhea and rectal bleeding—all of which Rachel began experiencing early on. By age 14, she had had her gall bladder removed and part of her large intestine taken out. She continues to undergo a number of painful treatments, including gastric-nasal feeding tubes and intravenous feeding methods that automatically exclude her from such normal teenage activities as going out for pizza or sleeping at the homes of friends. "I've tried to adapt as best I can," says this courageous teen, who, despite her Crohn's disease, volunteers to help others. "But it's extremely difficult for me to participate in lots of stuff. Sometimes I feel really separate from everyone else."

Despite suffering from a very severe case of Crohn's disease, 18-year-old Rachel Hettich is a straight "A" student, plays an active role in her community, and is planning her college career. Still, she looks forward to medical breakthroughs that "may not only treat the symptoms...but perhaps even cure the disease." The NIDDK supports a wide variety of research on Crohn's and other IBDs, with the hope of one day finding a cure for these debilitating diseases.

Complications of Crohn's disease include blockage of the intestines, as well as sores, or ulcers that tunnel through affected areas into surrounding tissue such as the bladder or skin. Arthritis, skin problems, inflammation in the eyes or mouth, kidney stones or gallstones, or liver disease also may occur. Currently, there is no cure for Crohn's, and the medications and treatments now available simply treat symptoms, but not the root cause of the disease, which remains elusive.

RACHEL HETTICH

To help the estimated 1 million people in the U.S. who suffer from Crohn's and other IBDs, the NIDDK supports a wide variety of research, with the hope of one day finding a cure for these disabling inflammatory bowel diseases.

NIDDK Crohn's/IBD Research Efforts

- *Funding of a new Inflammatory Bowel Disease Genetics Research Consortium to identify genes or genomic regions associated with increased risk of developing IBD. The consortium also will investigate age of onset, response to therapy, and susceptibility to environmental risk factors, as well as increase molecular understanding of IBD to open new avenues of research towards the development of novel therapies and new diagnostic methods.*
- *Continued funding of NIDDK's Digestive Diseases Research Centers, including support for centers that focus on IBD research and research training.*
- *Conducting workshops to discuss endpoints for clinical research in inflammatory bowel diseases.*
- *Supporting studies on progenitor cells of the gut and research relating to potential autoimmune and microbial influences in the development of IBD.*
- *Encouraging basic and clinical research into intestinal failure, "short gut syndrome," and intestinal transplantation.*

Living with the "Dark Beast"

According to Rachel's father, Bob Hettich, there is no evidence that anyone in either the immediate or extended biological family has ever suffered from Crohn's. "When Rachel was diagnosed 10 years ago, we had no idea what Crohn's disease was," he says. Today, the Hettich family refers to Crohn's as the "dark beast" and is committed to educating others about it. "Crohn's is very poorly understood," adds Mr. Hettich. "Most people don't realize how severe and serious this disease can be. Friends and relatives often say to us, 'Oh, we know someone with Crohn's and they didn't have much of a problem with it.'"

It's true that Crohn's affects people differently. Unfortunately, Rachel suffers from an extremely severe case. As a result, she has undergone every treatment possible over the years, including drugs, nutritional supplements, surgery, or at times a combination of all three. These treatments, however, only help control inflammation, correct nutritional deficiencies, and relieve symptoms like abdominal pain, diarrhea and rectal bleeding—and they come with their own sets of side effects and complications.

Anti-inflammatory drugs, for example, are often the first line of defense for people with Crohn's. However, over time, the body sometimes builds up resistance to these medications. People also can have unique reactions to medications. The medications Rachel takes for her Crohn's appear to result in "weird side effects," she says. "I've had bald spots on the back of my head the size of quarters," says Rachel, who thinks they were the result of one of her medications. "Also, when I perspire, it sometimes causes stains on my clothes that resemble bleach stains." Both of these apparent side effects are temporary, she says.

Many of the anti-inflammatory drugs taken by people with Crohn's also suppress the immune system, making them more susceptible to infection and other illnesses. "When I'm on these medications, I'll catch everything that's going around," says Rachel. "I can't tell if I'm suffering an ordinary stomach virus or an intestinal flare-up as a result of having Crohn's."

RACHEL HETTICH

To "rest" her intestines from these flare-ups, or because at times her intestines cannot absorb enough nutrition from food, Rachel is intravenously fed a special high-calorie liquid formula, called Total Parenteral Nutrition (TPN). Her family has been trained to place the formula into an IV bag. They then insert plastic lines from the bag into an IV line that has been surgically placed in either Rachel's chest or arms for what often amounts to a 10-hour infusion. "I did this for several years when I was in middle school," says Rachel. "Now that my anti-inflammatory medication is no longer working, I'll probably be doing TPN for six months." This will complicate Rachel's life immensely. "The treatment gives me more nutrition, and I have more energy," says Rachel, "but I can't stay out real late or have sleepovers at my friends' houses, and I miss lots of days from school." Even showering is problematic. "I have to put Saran wrap over the insertion, so it doesn't get wet," she adds.

Handling Life with IBD with Humor

In addition to having to endure all of the above, Rachel says that one of the worst things about living with Crohn's is its unpredictability. Intestinal flare-ups can occur at any time. Not only are they extremely painful for Rachel, but as a family the Hettichs are often left homebound until these episodes—which often can go on for weeks, sometimes months—subside.

"My family and I learned that if we did not maintain a positive, even somewhat humorous outlook on life, we would never survive," says Rachel. Over the years, therefore, the Hettich family developed its own version of the "top five ways that you know you have an inflammatory bowel disease." They are:

1. "Having a fever of 101.5 degrees is considered low grade."
2. "You consider the nurses at your doctor's office better friends than your school mates."
3. "Your school measures your absences in weeks rather than days."
4. "You plan all your activities and social life according to how many days it has been since your last infusion of an anti-inflammatory drug."
5. "You seriously consider putting a TV and mini-refrigerator in your bathroom."

Despite her chronic and severe disability, Rachel is a straight "A" student. "My guidance counselor and teachers are amazed at how well I keep up with my work, given the fact that I'm absent from school nearly half the time," she says. She plays an active role in her community by volunteering at a local animal shelter, working with Alzheimer's patients at a retirement home, and serving on the youth council at her church. And she has dreams for her future, as well. After graduating from high school Rachel plans to attend the University of Tennessee and major in anthropology. For all of these reasons, Rachel has been recognized as a "Local Hero" by the Crohn's and Colitis Foundation of America.

"I look forward to the future with great anticipation of medical breakthroughs that may not only treat the symptoms of Crohn's and other IBDs, but perhaps even cure the disease," Rachel says, with much hope of leading a more normal life as an adult.

(For medical information on IBD and ulcerative colitis, see

http://www.niddk.nih.gov/health/digest/pubs/crohns/crohns.htm

http://www.niddk.nih.gov/health/digest/pubs/colitis/colitis.htm)

STORY OF DISCOVERY

Genetic Insights into Pancreatitis and Pancreatic Cancer

When the pancreas produces enzymes to digest food, why don't those enzymes also digest the pancreas? Sometimes, they do—and with painful and potentially fatal consequences—as in the case of the disease hereditary pancreatitis. Several years ago, researchers discovered a mutation that abolishes one of the body's key safeguards against destruction of the pancreas by the very digestive enzymes it manufactures. This scientific breakthrough marked the beginning of a series of genetic discoveries that are providing new insights into hereditary pancreatitis, pancreatitis that arises for unknown reasons (idiopathic), and pancreatic cancer.

Patients with pancreatitis usually experience severe pain. As the pancreas becomes progressively injured and inflamed, in part as a result of infiltrating inflammatory cells, it no longer secretes enough enzymes into the small intestine for digesting food. Eventually, the pancreas cells that produce the vital hormone insulin can become damaged as well, leading to diabetes. Treatments exist to help manage the pain and digestive enzyme deficiency, but currently there are no cures or preventative therapies. Patients suffering long-term from pancreatitis are also at dramatically increased risk for pancreatic cancer. One of the most devastating of all malignancies, pancreatic cancer nearly always kills within a year of diagnosis, and often within six months.

Clinicians had long associated pancreatitis with alcoholism. While excessive alcohol consumption clearly plays a role in many pancreatitis cases, researchers recognized a hereditary form of pancreatitis as early as 1952. An attempt to find a hereditary pancreatitis gene in the 1970s, however, was unsuccessful. The identification of genes associated with pancreatitis awaited the advent of modern molecular and genetic technology.

In 1996, scientists found the first gene linked to a form of pancreatitis called hereditary pancreatitis, which generally strikes in childhood. This gene encodes the protein cationic trypsinogen, an inactive precursor form of the digestive enzyme trypsin. Trypsin helps digest proteins from food essentially by chopping them into pieces. To avoid digestion of the pancreas, trypsinogen normally does not become activated within the pancreas to form trypsin. If it does, the body has what scientists call a "fail-safe" line of defense: for the greater good, the prematurely-active trypsin commits molecular *hara-kiri*, slashing itself. Many people with hereditary pancreatitis have a particular mutation in the trypsinogen gene that disables this defense mechanism. Scientists have also identified other mutations in this gene. The continued identification of mutations that confer susceptibility to hereditary pancreatitis is useful for the design of diagnostic tests.

Among people whose genetic make-up predisposes them to hereditary pancreatitis, about one in five will not actually develop the disease. Surprisingly, researchers have even found pairs of identical twins in which one twin developed hereditary pancreatitis while the other did not, even though identical twins share the same chromosomal gene sequences and most types of environmental factors. These findings clearly suggest that other types of genetic factors (such as "epigenetic factors"), environmental factors, or chance events may also play a part in the development of hereditary pancreatitis. Scientists believe

STORY OF DISCOVERY

that pancreatitis results from a long chain of events activating different enzymes. In addition to known genetic influences, the disease is also precipitated by external factors such as food and alcohol. Gaining a better understanding of the complex interactions between different types of genetic and environmental factors will be a major challenge for future investigations.

Knowledge of genetic influences in hereditary pancreatitis will also help scientists assess environmental risk factors for pancreatic cancer, because people with hereditary pancreatitis are at increased risk for this cancer. Scientists recently found that pancreatic cancer develops an alarming 20 years earlier in hereditary pancreatitis patients who smoke. It is not yet clear whether smoking also has this effect in people who don't have hereditary pancreatitis.

Several years after the discovery that mutations in the trypsinogen gene cause hereditary pancreatitis, scientists identified mutations in a different gene that are associated with idiopathic pancreatitis. This gene encodes a protein called SPINK1, which normally helps protect the pancreas by inhibiting the digestive functions of prematurely-activated trypsin. However, the effects of *SPINK1* mutations are subtle, and dissecting the nature of their association with pancreatitis remains challenging.

The identification of another gene associated with idiopathic pancreatitis had its origins in research on a seemingly unrelated disease, cystic fibrosis, which is caused by mutations in the *CFTR* gene. *CFTR* function is important in many organs, including the pancreas, and scientists recently found that many idiopathic pancreatitis patients harbor a particular pattern of *CFTR* mutations.

With the discovery in 1996 of the link between trypsinogen and hereditary pancreatitis and the findings in 1998 and 2000 that *CFTR* and *SPINK1* are associated with idiopathic pancreatitis, it would seem that another major genetic discovery in pancreatic disease might arrive in 2002. One did. Investigators have now brought to light the first genetic defect specific to pancreatic cancer, pinpointing a region on chromosome 4 as likely to contain a pancreatic cancer susceptibility gene. The future identification of this gene will enhance our understanding of pancreatic cancer and provide a potential tool for early diagnosis.

Screening patients for genetic mutations can have many health benefits, such as alerting patients at risk to seek early medical intervention. However, the results of a genetic test may also influence reproductive choices and the ability to obtain health or life insurance. Deeply concerned about the ethical and social implications of genetic testing for patients and their families, investigators recently surveyed individuals participating in a hereditary pancreatitis genetic research study. The most common reasons the participants gave for joining the study were to help family members and future generations and to obtain genetic testing. The major concern they expressed was the fear of insurance discrimination. The most common reasons for sharing their results were to provide medical information to their families and to improve their own medical care.

These achievements in research on pancreatitis and pancreatic cancer not only illuminate genetic influences underlying these diseases, but also will facilitate research on environmental factors that contribute to disease in genetically-susceptible individuals. Already, the identification of hereditary pancreatitis mutations has led to the development of gene-based methods to evaluate a person's risk for this disease. Further understanding of genetic factors associated with different forms of pancreatitis and pancreatic cancer will undoubtedly lead to new strategies for diagnosis, treatment, and prevention.

VISION STATEMENT

Jeffrey Gordon, M.D.

A Vision of the Future in Digestive Diseases Research: Life in a Microbial World

Dr. Jeffrey Gordon is Professor and Head of the Department of Molecular Biology and Pharmacology at the Washington University School of Medicine in St. Louis. He joined the faculty at Washington University in 1981, after completing his clinical training in internal medicine and gastroenterology and after serving as a research associate in the Laboratory of Biochemistry at the National Cancer Institute. He has remained at Washington University for his entire professional career. In 2001, he was elected to the prestigious National Academy of Sciences. When asked to present his future vision to the NIDDK Advisory Council in September 2002, Dr. Gordon shared his enthusiasm for an emerging field within digestive diseases research that he finds inspiring: the study of "something that is with us all of our lives—our affiliated microbial communities."

Dr. Jeffrey Gordon

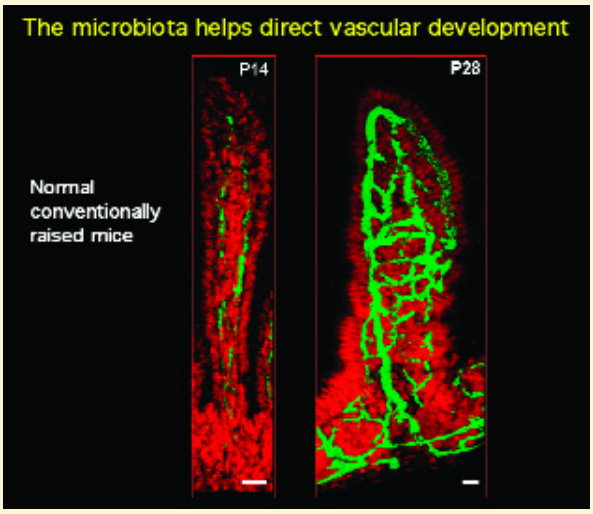
A Transcendent View of Our Genes, Development, and Health

"Beginning at the moment of our birth, we become colonized by a remarkably complex, dynamic, and abundant society of microorganisms," Dr. Gordon explained. In fact, in our intestines we play host to 500 to 1,000 species of bacteria. Dr. Gordon proposed a comprehensive genetic view of ourselves as a life-form that encompasses not only our own genome, but also the "microbiome"—the collective genomes of all of our affiliated microbial partners. We currently know very little about how components of these microbial communities (microbiota) influence our post-natal development and adult physiology—but they do, as scientists like Dr. Gordon are discovering—and in extraordinary and surprising ways. Dr. Gordon depicts these microorganisms as "master physiologic chemists" that, through co-evolution with humans, have developed "very clever chemical strategies for regulating our gene expression (influencing whether genes are active or not) in ways that benefit both them and us." Dr. Gordon's vision of the future of research includes identifying the microbial genes and gene products that modulate the expression of our genes, and determining which of our genes are affected. Additionally, interactions between normal gut bacteria and their hosts may predispose susceptible individuals to a range of diseases both within and outside of the gastrointestinal tract. "Inflammatory bowel disease is one often-cited example of how deranged interactions between indigenous microbes and us can lead to immunopathologic states," Dr. Gordon noted, but he added that other diseases, including irritable bowel syndrome, gastrointestinal cancer, and metabolic disorders such as obesity and diabetes may also arise in part through disruption of normal host-microbial interactions. Research on our intestinal microbiota should provide new molecular targets for drug development and new chemical entities for preventing and treating diseases.

VISION STATEMENT

Technologies for Studying the Microorganisms in Our Intestines

Because of the "almost unimaginable degree of complexity" of the interactions between gut microbes and their hosts—and among the microbes themselves—Dr. Gordon's laboratory devised a clever way to simplify these interactions for the purpose of experimental analysis. The scientists first raised mice under germ-free conditions so the animals had no intestinal microorganisms. Then, the scientists added back defined microbial populations to some of these mice, and evaluated the effects, comparing the newly-colonized animals with mice that were maintained in an entirely germ-free environment. As a model microbe, they chose *Bacteroides thetaiotaomicron (B. theta). B. theta* is a prominent member of the normal intestinal microbiota of both mice and humans, and critically for their experiments, *B. theta* can be readily grown in the laboratory and genetically manipulated—unlike many of the other microbes that reside in the gut. Further, Dr. Gordon pointed out, *B. theta* normally colonizes the gut during a critical phase of post-natal gut development, the suckling-weaning transition. This timing affords the scientists an opportunity to see how a component of the microbiota may influence intestinal maturation. To examine gene expression in different cellular components of the intestine, Dr. Gordon's laboratory has employed an advanced technique called laser capture microdissection, which enables them to isolate cells from precise areas of the intestine. Material can then be obtained from the harvested cells to analyze which genes are affected by the presence or absence of *B. theta,* or other gut microbes. His laboratory has used the power of DNA microarray technology and bioinformatic methods to obtain a comprehensive analysis of *in vivo* cellular responses to bacterial colonization.



The indigenous microorganisms (microbiota) in the gut help direct the development of the intestinal blood vessels (vascular system). The photo on the left shows a section of mouse intestine taken 14 days after birth; the vascular system is stained with green fluorescent dye. By the 28th day after birth, as shown in the photo on the right, a very elaborate intestinal vascular system has developed in conventionally-raised mice. This normal vascular developmental program is not completed in mice raised in a special germ-free environment.

Work of the "Master Physiologic Chemists" in the Gut—Influencing Intestinal Biology and Architecture

One of the intriguing lessons learned from the experiments of Dr. Gordon's laboratory is that *B. theta* and other components of the normal microbiota can fortify the mucosal barrier that lines the gut; this barrier prevents intestinal bacteria from escaping into other parts of the body. Moreover, although disease-causing microbes usually elicit an inflammatory response when they infect us, *B. theta* manages to colonize the gut without eliciting such a reaction. From their experiments in mice, Dr. Gordon's laboratory found that *B. theta* does not affect the expression of host genes that would be involved in an inflammatory response—an important feature for an organism that must establish and maintain a long-standing symbiotic

relationship with its host. A deeper understanding of how our indigenous microbes manage to live peacefully in the gut may provide clues as to why, in some circumstances, the immune system aberrantly mounts an inflammatory response to these microorganisms. Interestingly, research from Dr. Gordon's laboratory also revealed that *B. theta* regulates the expression of a host bactericidal protein, angiogenin-4. "In essence," Dr. Gordon said, "components of the microbiota are able to regulate expression of endogenous antibiotics and help define the microbial ecology of their host niche."

The microbiota additionally helps direct post-natal blood vessel development in the intestine. In mice, between about 2-to-4 weeks after birth, the system of tiny blood vessels (microvasculature) in the intestine becomes very elaborate, allowing for growth of the gut and efficient absorption of nutrients. Also during this time interval, components of the microbiota assemble and the composition, complexity and density of the intestinal bacterial community increases dramatically. In mice raised germ-free, the program of intestinal capillary development is arrested at an early stage. Strikingly, when the scientists retrieved gut microbes from a conventionally-raised animal and gave them to mice that had been raised germ-free, the formerly germ-free animals completed their program of blood vessel formation in just 10 days. Administering *B. theta* alone to mice raised germ-free also catalyzed completion of microvasculature development. They went on to show that microbial regulation of blood vessel formation is dependent upon signals that are processed by Paneth cells—a type of intestinal epithelial cell that is a key component of the gut's innate immune system. These findings illustrate the importance of considering features of post-natal mammalian development as manifestations of mutually beneficial collaborations with microbes.

Further Activities of Gut Microbes: The Microbiology of Human Nutrition and Obesity

Another service that components of the microbiota provide their host—in exchange for a place to live and a food supply—is help with processing and uptake of foodstuffs we eat. Dr. Gordon refers to the microbiota as a "multi-cellular symbiont that lives in harmony with us, and facilitates our nutrient processing and uptake." For example, we are not capable of digesting many of the plant polysaccharides that we eat—we don't have the necessary genes. We rely on enzymes manufactured by the bacteria in our intestines for this task. Dr. Gordon's group has recently finished sequencing the entire *B. theta* genome. The results show that this symbiont has a vast repertoire of genes encoding proteins that are able to capture undigested polysaccharides from gut lumen and break them down. It also has an elaborate system for sensing the luminal environment. This well-developed sensory apparatus probably gives it a competitive advantage so that it can become a predominant member of the densely populated intestinal ecosystem. Further, experiments involving *B. theta* colonization of the germ-free intestine showed that it can induce expression of host genes encoding proteins that facilitate absorption of sugars, and that help metabolize fats. As components of the microbiota mediate efficient extraction of nutrients—and associated calories—from food, they may be one determinant of whether their host has a predilection towards obesity. "Germ-free mice must consume 30 percent more chow in order to maintain their body weight compared to their conventionally-raised counterparts," explained Dr. Gordon. He suggested that the capacity to absorb nutrients may vary between individuals as a function of the composition of their microbiota. Thus, the microbiota may be one predisposing environmental factor for obesity." Dr. Gordon hypothesized that the intestinal bioreactor may be more efficient at extracting calories from the diet in obese compared to lean individuals.

VISION STATEMENT

The Future of Research on the Microbial World Within Us

We still know relatively little about the biodiversity within the human intestine—both in healthy individuals and those with diseases. Thus, Dr. Gordon stressed the importance of future research to enumerate the components of the intestinal microbiota. In addition to understanding the activities of the individual microbes, it will be important to learn how the components of the microbiota interact with one another to establish and maintain a microbial community. The knowledge emanating from such research should enable scientists to "explore hypotheses about whether changes in the composition of the microbiota are directly associated with a variety of diseases." Moreover, such knowledge would allow scientists to design and carry out experiments in which microorganisms are administered as potential therapeutic agents (referred to as probiotics). Dr. Gordon further stressed the importance of comparative microbial genomics to identify the genetic features that define the capacity for symbiosis (and distinguish symbionts from pathogens). He called for a "microbiome sequencing project," that would complement the sequencing of the human genome.

A "Transcendent View of a University"

In concluding the presentation of his view of the future of research, Dr. Gordon put forth a perspective about scientific interactions within research institutions. A problem such as the molecular foundations of gut symbiosis and its regulation of postnatal development and adult physiology demands that investigators cross many disciplines. "It is critical that students and their mentors, departments, and universities, together with funding agencies such as the NIH, learn to operate at a number of interfaces." Dr. Gordon described three such interfaces: the biological and physical-computational sciences, the biological and chemical sciences, and the biological and clinical sciences. If we can do this, Dr. Gordon said, "We will be able educate the next generation of leaders in our fields, and attack major problems related to human health."

PATIENT PROFILE

Chris Klug

Liver Transplant Gives Olympian More than Just a Shot at the Gold

For a world-class athlete like 30-year-old Chris Klug, the abbreviation "PSC" should signify "Professional Snowboarding Champion." But the American Olympian who won the bronze medal in the giant slalom event at the 2002 Games in Salt Lake City is well aware that PSC actually stands for "Primary Sclerosing Cholangitis," a rare and potentially deadly liver disease. Had it not been for the liver transplant Chris received in July of 2000, he may never have made it to the Olympics, let alone taken the bronze. "I realize what it's like to receive the gift of life and what it means to have a second chance," says Chris. "I intend to make the most of it, and to continue spreading the life-saving message of organ donation." Chris received a life-sustaining liver transplant only because a family he has never met decided to give the "gift of life" and allowed the liver to be taken from a loved one who had just died.

"Organ donors are heroes. I am here today because a family said 'yes' to a second chance for me to pursue my dreams. I'm forever grateful to the donor and to his or her family."

Chris waited a year-and-a-half before receiving a cadaveric liver for his transplant. "They tell me that 16 people die each day while on the transplant waiting list," says Chris. "I thought I might be one of them." But a new study, sponsored by the NIDDK, called the Adult-to-Adult Living Donor Liver Transplant Cohort Study (A2ALL), may point the way towards reducing the waiting time, as well as the risk of liver transplantation for patients like Chris and the thousands of others in need of a liver transplant.

Thirty-year-old Chris Klug won the bronze medal in the giant slalom event at the 2002 Olympic Games, less than two years after undergoing surgery to receive a liver transplant. "I just realize how lucky I am to be out here doing what I love to do—traveling the world on my snowboard." Photo: Tom Zikas Photography

Living with PSC

Chris' ordeal began long before he received his liver transplant. He was diagnosed with PSC at age 21 during a routine physical. It wasn't a diagnosis anyone would wish for, but the fact that it was diagnosed early probably saved Chris' life. Legendary Chicago Bears football running back Walter Payton, on the other hand, was not so fortunate. Payton died because PSC wasn't discovered until it was too late to treat it effectively.

CHRIS KLUG

In PSC, the bile ducts that drain bile from the liver become inflamed, scarred, and eventually blocked. When the ducts are completely blocked, bile builds up in the liver and damages liver cells, leading to scarring of the liver, cirrhosis and ultimately liver failure. Researchers do not yet know what causes PSC. Among the theories under investigation are the role of bacteria and viruses, and problems in the immune system. PSC is often associated with ulcerative colitis, a type of inflammatory bowel disease.

Prior to his diagnosis, Chris had no warning signs that anything might be wrong. "I had a routine physical just before the 1992 World Cup competition season, and some strange numbers came back from my blood tests," says Chris. Even after he was diagnosed, Chris says he felt like "a million bucks," and that he often wondered if "they had the right person" with respect to his diagnosis. Yet this is typical for PSC. It usually begins between the ages of 20 and 50 and more often affects men than women. A person can have the disease for years before he or she feels bad in any way. The usual first symptoms are itching, fatigue, jaundice, and episodes of chills and fevers.

"After I was diagnosed, there was always this huge unknown," says Chris. "I never really knew if common cold symptoms were something I should pay attention to or if they simply indicated a common cold. I sometimes freaked over nothing." By Spring of 2000, however, his disease began to affect the way he felt and his athletic performance. The PSC caused anemia, a decrease in the number of red blood cells, which are responsible for delivering oxygen to the body. "I tried to maintain my workouts," says Chris, "but I just didn't have the energy or oxygen capacity." Eventually, he also began having pains in his side over the liver "as if someone had jabbed me with a dagger." After having an endoscopic retrograde cholangiopancreatography, or ERCP, a procedure which enables physicians to visualize an outline of the gall bladder and bile ducts in the liver, Chris was informed that his liver was severely scarred and approaching irreversible liver failure, known as end-stage liver disease. "I was told it was time to get serious about finding a new liver, meaning a transplant," says Chris.

Waiting for a New Liver

For Chris, and for many other transplant candidates, the hardest part is waiting for an organ to become available. Chris was on the waiting list for about a year-and-a-half. "I wore a pager every minute of the day and carried a cell phone as a backup in anticipation of receiving a call informing me that a liver was available that matched my blood type and age," says Chris. Once again, Chris was fortunate. "When I finally got the call, I was relieved that the wait was over, but scared to death of the prospect of possibly not surviving the surgery." Not only did Chris survive the surgery, but a month after his transplant he was back in the gym doing rehabilitation therapy, and three months after that was back on the World Cup snowboarding circuit.

"They tell me that 16 people die each day while on the transplant waiting list," says Chris. "I thought I might be one of them."

Chris credits his extraordinary recovery from surgery to lots of prayers, a great team of doctors and nurses, a donor family that said "yes," a perfect donor match, and his physical and mental preparation. Chris says, "In essence I trained for the transplant, both mentally and physically." Today, Chris remains on daily doses of medication to keep his immune system from attacking and rejecting his transplanted liver as "foreign" tissue, medication he will need to take for the rest of his life. Chris says, "except for colds that seem to last forever," he doesn't experience any serious side effects from the medication. "I just realize how lucky I am to be out here doing what I love to do—traveling the world on my snowboard," he adds.

CHRIS KLUG

Adult-to-Adult Living Donor Liver Transplant Cohort Study (A2ALL)

Over the last 20 years, liver transplantation has become the standard of care and the only cure for end-stage liver disease. Now more than 4,000 transplants are performed yearly. However, there are at least 17,000 patients like Chris on the transplantation list awaiting cadaveric liver donations. As the waiting list has expanded, waiting time also has grown, resulting in an increase in the numbers of patients who die while waiting.

Living donor liver transplantation is an alternative to cadaveric transplantation. By providing valuable information on the outcomes of living donor liver transplantation, NIDDK's A2ALL will assist physicians, patients, and potential donors in making life-saving transplant decisions. Use of living donors helps to avoid the lengthening waiting period for cadaveric transplant, as well as greatly reduce the ischemic period, or the time between removing the organ from the donor and transplanting it into the patient. It also allows more time for evaluation of the donor, and changes the operation from an emergency into a scheduled procedure.

By helping physicians, patients, and potential donors make life-saving transplant decisions, the NIDDK's Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) has the potential for changing the face of liver transplantation.

The major issue related to living donor liver transplants is that it is a difficult and potentially dangerous operation for the donor. More than half of the liver must be removed to provide enough liver for the transplant candidate. Even in the best hands, death of the donor can occur and its estimated rate is 1 in 250 donor operations. Living donor liver transplantation also provides the recipient with a smaller portion of liver than would have been received with cadaveric transplantation. The A2ALL study will provide a scientific, balanced, and unbiased evaluation of living donor liver transplantation, both its risks and its benefits, and help to insure optimal safety in this most challenging of all liver operations.

Chris' physician, Gregory Everson, M.D., is also a co-investigator for the NIDDK-A2ALL study. "The impact of the study on the current and future waiting list for liver transplantation will depend upon several factors, including living donor recipient outcomes, the donor's safety, and other factors, such as immunosuppression and recurrent hepatitis C infection, which may have specific issues in living donor liver transplant cases," says Dr. Everson. (Hepatitis C, a virus that targets liver cells, is a leading cause of liver failure and can reemerge in patients post-liver-transplant, attacking the healthy new liver tissue.) He adds that the current criteria established for living donor liver transplantation address the needs of only five to 15 percent of people on the organ waiting list, and may need to be expanded.

Even with these caveats, "This study has the potential to establish living donor liver transplantation as a viable alternative to standard cadaveric transplants for future potential recipients and, thereby, change the face of liver transplantation," says Dr. Everson.

Whether through living donor or cadaveric transplantation, the fact is that organ donations save lives. Just ask Chris Klug. "To receive the gift of life is a humbling experience, and I will be forever grateful for my second chance," says the extremely talented athlete who is extremely happy to be alive.

Each kidney contains about 1 million nephrons, which are the working units of the kidneys. Nephrons remove wastes and excess fluids from the blood. Illustration: Maryetta Lancaster, for NIH Medical Arts and Photography Branch.

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.

Chronic kidney disease is a growing epidemic in the U.S. It often progresses to irreversible kidney failure, which requires treatment with dialysis or kidney transplantation for patient survival. Presently, it is estimated that from 10 to 20 million Americans have substantially impaired kidney function. Diabetes and, to a lesser extent, high blood pressure are the main causes of kidney disease, accounting for up to 70 percent of all new cases of chronic kidney disease. The epidemic is due in large part to the increase of type 2 diabetes in the U.S.

The U.S. has seen an enormous increase in people with end-stage renal disease (ESRD). In the year 2000, almost 100,000 people had progressed to ESRD, with the result that a total population of about 300,000 patients with ESRD was sustained on dialysis, while an additional 80,000 people had functioning transplanted kidneys. These numbers have doubled since 1990 and are expected to nearly double again by 2010. The cost of ESRD is high—almost $18 billion for healthcare alone in 1999, as well as $2 billion to $4 billion of lost income for patients.

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

The NIDDK devotes considerable resources to understanding the basic mechanisms underlying the causes and progression of kidney disease to ESRD. The Institute's efforts to combat ESRD include research to reduce morbidity and mortality from bone, blood, nervous system, metabolic, gastrointestinal, cardiovascular, and endocrine abnormalities in ESRD, and to improve the effectiveness of dialysis and transplantation. Major areas of research focus include identification and testing of possible therapeutic interventions to prevent development or halt progression of kidney disease, and identification of the risk factors for ESRD and cardiovascular disease. A major new outreach initiative is the National Kidney Disease Education Program.

Urologic diseases affect persons of all ages, result in significant health care expenditures, and, if improperly diagnosed or improperly treated, may lead to irreversible kidney and/or bladder damage and possibly death. Nonmalignant urologic diseases include benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, and congenital anomalies of the genitourinary tract.

Benign prostatic hyperplasia affects one half of men age 51 to 60, and 90 percent of men past age 80. Prostatitis is inflammation of the prostate gland that accounts for a significant percentage of all office visits by young and middle-aged men for complaints involving the genital and urinary systems.

In 1997, urinary tract infections accounted for well over 8 million physician visits at a cost of $1 billion. Urinary tract stone disease, commonly referred to as kidney stones, accounted for over 1.3 million physician visits in 1997. Interstitial cystitis (IC) is a debilitating, chronic bladder disorder, which has been estimated to affect as many as 1 million Americans, 90 percent of whom are women. In 1998, IC was estimated to cost about $1.7 billion in medical expenses and lost wages. About 13 million Americans, most of them women, suffer from urinary incontinence. In 1995, the "societal" cost of urinary incontinence was estimated to be $26.3 billion for individuals 65 and older. Genitourinary tract abnormalities are the most common birth defect. One such abnormality, vesicoureteral reflux, is one of the most common causes of kidney failure in children, occurring in an estimated 1-to-2 percent of newborns.

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

The Institute's hematology research program emphasizes a broad approach to understanding the normal and pathologic function of blood cells and the blood forming system. Major areas of interest include diseases such as sickle cell anemia, thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, and thrombocytopenia. The Institute has issued several research solicitations recently to emphasize research on the biology and genetic regulation of stem cells. Stem cells are crucial to the eventual broad application of gene therapy and for improved transplantation of bone marrow cells. An additional area of long-term priority has been the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for diseases such as Cooley's anemia (thalassemia major).

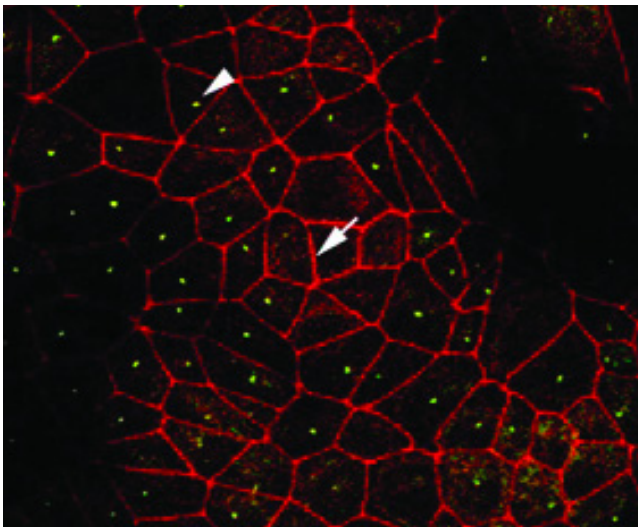
CILIA AND POLYCYSTIC KIDNEY DISEASE

Polycystic kidney disease (PKD) has been estimated to affect as many as 500,000 to 600,000 children and adults in the U.S. PKD is characterized by massive enlargement of the kidneys due to the presence of fluid-filled cysts and is the fourth leading cause of kidney failure. Most patients with the most common form of PKD, ADPKD, have a mutation in either one of two genes, *PKD1* or *PKD2*. Recently, the gene causing ARPKD, a less common but more lethal form of PKD, was identified.

The proteins encoded by *PKD1* and *PKD2*, polycystin-1 and 2, respectively, can form a functional complex that may be involved in cell signaling in kidney epithelial cells. Versions of genes very similar to human *PKD1* and *PKD2*, gene "homologs," have been found in the genomes of a number of model organisms, from worms to mice. In mice, researchers have also found defects in a number of other genes that can cause PKD. All of these discoveries have propelled research to figure out how disruptions in PKD-related genes derail normal kidney tissue development or maintenance to cause cyst formation and progressive renal (kidney) disease.

Fascinating new studies in model organisms suggest that mutations in PKD-related genes cause structural or functional defects in cilia, which in turn contribute to the development of PKD. Cilia are hair-like projections found on the surface of cells in certain tissues. They are composed of cell membrane stretched over a protein "scaffold," and serve many different functions. Cilia are used by some cells to sweep particles in a polarized direction, as in the cilia on cells lining the trachea (windpipe). Other cells use cilia as "antennae" to sense and respond to changes in the extracellular environment.

NIDDK-supported researchers studying gene homologs of human *PKD1* and *PKD2* in the nematode worm *C. elegans*—*lov-1* and *pkd-2*, respectively—recently found evidence that the two genes function in the same cellular pathway, similarly to human *PKD1* and *2*. *C. elegans* doesn't have kidneys,



In polarized kidney epithelial cells grown in culture (outlined in red), proteins involved in polycystic kidney disease (PKD), such as polycystin-1, can be found in the cell cilia (yellow). Photo: Dr. Bradley Yoder and Dr. Lisa Guay-Woodford. From The Journal of the American Society of Nephrology, Vol 13, 2002, 2508-16. Reprinted with permission from Lippincott Williams & Wilkins.

however. Instead, both *lov-1* and *pkd-2* are necessary for proper male worm mating behavior—which requires sensing environmental cues. The researchers found that the *C. elegans* PKD-2 protein, like the LOV-1 protein, appears to be exclusively expressed in male-specific sensory neurons used in mating—which are also ciliated. Furthermore, both LOV-1 and PKD-2 proteins are enriched in the cilia.

As it turns out, polarized kidney epithelial cells, specialized cells that line both normal kidney tubules and PKD cysts, are also ciliated, possessing a single cilium per cell. The function of this cilium is unknown, but there is evidence that it may fulfill a sensory function, possibly detecting changes in fluid flow in the kidney. Previously, researchers had observed that mutations in the gene coding for "polaris," a PKD-related protein in mice, disrupt cilia formation in polarized kidney epithelial cells. In a recent study, NIDDK-supported researchers identified and characterized the *cpk* gene, which is linked to PKD in one mouse model of the disease. The *cpk* gene is expressed primarily in mouse kidney and liver and encodes cystin, a novel small protein. When the researchers engineered an easily detectable version of cystin and expressed it in polarized kidney epithelial cells grown in a culture dish, they observed it in the cilium—similarly to polaris.

But are polycystin-1 and polycystin-2, the proteins directly implicated in human PKD, also localized to kidney cell cilia? The two proteins had already been detected in other parts of these cells, including specialized patches of cell membrane and in cellular organelles. NIDDK-supported researchers re-examined these cells and found that, indeed, mouse polycystins-1 and -2, like cystin and polaris, can be found in the cilium of polarized kidney epithelial cells grown *in vitro*. Furthermore, another NIDDK-supported research team recently examined what the PKD proteins may be doing in the cilium. They found that when they first blocked the activation of polycystin-1 or polycystin-2 on cultured kidney epithelium cells and then used fluid flow to bend the cells' cilia, the cells no longer engaged in normal signaling pathways in response to this mechanical stress. These results suggest that the presence of polycystin-1 and polycystin-2 in the cilium is necessary for fluid-flow sensation, which may in turn help regulate tissue growth and change in the kidney.

The roles of the kidney epithelial cell cilium in normal development or maintenance of polarized kidney epithelium—and hence, its possible role in cyst formation—are still under investigation. The results of these studies now suggest, however, that proper function of the cilia and at least some of its associated proteins may be necessary to prevent PKD—a significant new twist in our understanding of PKD. Continued investigation of both cilia themselves and the PKD-related proteins is required to determine exactly how defects in the kidney epithelial cell cilium may contribute to the pathogenesis of PKD.

Barr MM, DeModena J, Braun D, Nguyen CQ, Hall DH and Sternberg PW: The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. *Curr Biol* 11: 1341-6, 2001.

Hou X, Mrug M, Yoder BK, Lefkowitz EJ, Kremmidiotis G, D'Eustachio P, Beier DR and Guay-Woodford LM: Cystin, a novel cilia-associated protein, is disrupted in the *cpk* mouse model of polycystic kidney disease. *J Clin Invest* 109: 533-40, 2002.

Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AEH, Lu W, Brown EM, Quinn SJ, Ingber DE and Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cell. *Nat Genet* (Advanced Online Publication 6 January 2003).

Yoder BK, Hou X and Guay-Woodford LM: The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol* 13: 2508-16, 2002.

UNDERSTANDING AND PREVENTING KIDNEY DISEASE PROGRESSION

Chronic kidney disease affects an estimated 10 to 20 million Americans. It can result from a number of kidney diseases, including polycystic kidney disease (see preceding section) and focal segmental glomerulosclerosis, or as a secondary complication of other diseases or conditions, such as diabetes and hypertension (high blood pressure; see also "Vision Statement—Robert Schrier, M.D."). Exposure to toxins or high-protein diets in some cases can also contribute to kidney problems and the development of chronic kidney disease.

Chronic kidney disease can progress slowly over many years, and patients are often unaware of the condition until they have advanced loss of kidney function. In its most severe stage, chronic kidney disease develops into end-stage renal disease (ESRD)—irreversible kidney failure. The factors underlying kidney disease progression are not well understood. Genetics, environment, and nutrition are all thought to contribute to a greater or lesser degree. Because chronic kidney disease is linked to higher risk for cardiovascular disease (see "Story of Discovery—Chronic Kidney Disease and Cardiovascular Disease") and can ultimately progress to kidney failure, it is imperative to more fully understand the factors driving progression of disease.

Identifying Novel Genes Involved in Kidney Disease Progression: A better understanding of the large number of genes expressed in normal and diseased kidneys and how they interact should help clarify how kidney disease begins and how it progresses. Serial analysis of gene expression (SAGE) is a technique that allows researchers to generate a relatively comprehensive profile of the large number of genes expressed in a particular cell type or tissue. These "snapshots" of global patterns of gene expression may allow scientists to better define cell biology at the molecular level without making assumptions *a priori* about which genes might be important. This approach can be valuable in identifying novel targets for therapy that might otherwise not be considered.

One recent application of SAGE has been the comparison of gene expression profiles in the kidneys of two different strains of mice, ROP and C57Bl/6, that exhibit divergent physical manifestations of the same genetic defect. When present in the ROP strain of mice, this defect, known as Os/+, causes skeletal abnormalities; a 50 percent reduction in the number of nephrons, the tiny filtration units in the kidney; and progressive kidney damage that resembles the human disease focal segmental glomerulosclerosis. However, in the C57Bl/6 strain of mice, Os/+ produces the same skeletal defects and similar kidney defects, but not progressive kidney disease.

Using SAGE, NIDDK-supported researchers recently identified 63 genes whose expression was significantly different between the two mouse strains. Thirty-eight of these gene products were more abundant in the sclerosis-prone ROP-Os/+ mouse kidney, including antioxidant genes involved in stress response. Gene products relatively under-represented in the ROP-Os/+ kidney included ones important in the maintenance of normal cell structure and function. By using this approach, researchers now have clues about genes and pathways that may play a role in the progression of kidney disease that might not have been expected or anticipated using approaches focusing narrowly on the Os/+ defect.

Finding Factors That Contribute to Kidney Disease Progression—Clinical Efforts: It is imperative to discover the factors that contribute to the decline in kidney function and the development of cardiovascular disease in people with chronic renal insufficiency. Further research is needed before interventions can be evaluated and implemented. To date, few studies have focused on people with chronic kidney disease before they reach ESRD.

One type of study that has played an important role in defining risk factors for a wide-range of diseases is the prospective cohort study. To determine the risk factors for rapid decline in kidney function and development of cardiovascular disease, the NIDDK recently established the Chronic Renal Insufficiency Cohort (CRIC) Study. This is a seven-year prospective, multi-ethnic, multi-racial study of approximately 3,000 patients with chronic kidney disease. Participants will reflect the racial, ethnic, and gender composition of the U.S. ESRD patient population. The data and samples obtained from people in this study will serve as a national resource for investigating chronic kidney disease and cardiovascular disease. Establishing this cohort of patients and following them prospectively will also provide researchers with an opportunity to examine genetic, environmental, behavioral, nutritional, quality-of-life, and health resource utilization factors in this population. Five geographically diverse centers are participating in the study. The CRIC study began protocol development in September 2001, and enrollment for the study is expected to begin in Spring 2003.

Another clinical study supported by the NIDDK, the Continuation of AASK Cohort Study, focuses specifically on defining factors contributing to kidney disease progression in African Americans. African Americans are disproportionately afflicted with ESRD: although they constitute approximately 12 percent of the U.S. population, African Americans comprise 32 percent of the prevalent ESRD population. The Continuation of AASK Cohort Study commenced at the conclusion of the African American Study of Kidney (AASK) Disease and Hypertension. A landmark clinical trial, the AASK study demonstrated that persons with kidney disease caused by hypertension (high blood pressure) have a better chance of reducing the risk of kidney failure if they take an angiotensin converting enzyme inhibitor medication. The primary goal of the continuation study is to investigate environmental, socio-economic, genetic, physiologic, and other factors that influence progression of kidney disease in a well-characterized cohort of African Americans with hypertensive kidney disease.

Preventing Progression of Kidney Disease: The NIDDK is supporting a number of studies and clinical trials that are evaluating therapeutic interventions to prevent kidney disease progression. One new study, the Focal Segmental Glomerulosclerosis (FSGS) clinical trial, will examine interventions to prevent progression of this disease. FSGS causes scarring in the kidney and is a major cause of renal disease in children and young adults. It can also recur post-kidney-transplant leading to kidney transplant injury or loss. The NIDDK has also established a Polycystic Kidney Disease (PKD) Clinical Trials Network to design and implement clinical trials of agents that might slow progressive loss of kidney function in PKD, the fourth leading cause of ESRD.

Another extremely important aspect of intervention in chronic kidney disease is increasing public awareness. The NIDDK recently launched the National Kidney Disease Education Program (NKDEP) to educate and inform the public about the risks for and complications of chronic kidney disease, and ways that disease can be slowed or even prevented (see sidebar, "The National Kidney Disease Education Program"). The NKDEP has begun a pilot campaign in four U.S. cities, targeting health care providers, patients, and insurers with a message focused on identifying risks, patient screening, and appropriate treatment. The initial campaign is especially focused on African Americans, a patient group at particularly high risk for kidney disease. In later phases, the campaign will extend to other minority groups at high risk.

Ultimately, the goal of this educational campaign is to reduce complications and death due to kidney disease and kidney failure among all Americans.

Schelling JR, El-Meanawy MA, Barathan S, Dodig T, Iyengar SK and Sedor JR: Generation of kidney transcriptomes using serial analysis of gene expression. *Exp Nephrol* 10: 82-92, 2002.

NEW KNOWLEDGE IN DIALYSIS

The introduction of hemodialysis in the early 1960s has saved the lives of countless persons suffering from kidney failure, also called end-stage renal disease (ESRD) (see sidebar, "Lasker Awardees—Dr. Willem Kolff and Dr. Belding Scribner"). Normally, the kidneys remove toxic by-products of metabolism, such as urea and creatinine, for excretion in urine, and maintain salt balance in the blood. These functions are lost in ESRD. In hemodialysis, these functions are approximated by circulating a patient's blood through a specialized external filter to remove toxins and then returning it to the body. ESRD patients typically undergo this procedure at least three times a week, for 2-to-4 hours at each session. This enables patients to survive as they await the only cure for ESRD—a kidney transplant.

Unfortunately, despite the success of hemodialysis, it is far from perfect. Mortality on hemodialysis is still high—current projections are 70 percent mortality within 5 years of starting dialysis. Moreover, quality of life is poor. The dramatic rise in diabetes, the leading cause of ESRD, has fueled the increasing number of persons on dialysis. In turn, the average time a person remains on dialysis has increased, because the number of kidneys available for transplant has become further outstripped by the need. Also noteworthy is that the population receiving maintenance hemodialysis now, as compared to three decades ago, is older and has more co-morbid conditions to contend with, which may be adversely affected by the current limitations of dialysis.

To address these issues, the NIDDK sponsored a major clinical trial, the HEMO study, to carefully evaluate whether patient survival on dialysis could be improved either by making dialysis doses more intense, or by using a different type of dialysis filter—a "high-flux" filter that can remove larger waste particles from the blood. This study enrolled more than 1,800 patients, who were randomly assigned to standard or high dialysis doses with low- or high-flux filters. The HEMO study investigators found that, on average, treatment under the dialysis guidelines currently in use provides essentially the same benefits as more intensive regimens.

The HEMO study is the most comprehensive, randomized clinical trial to date to evaluate the efficacy of hemodialysis protocols. However, researchers are still investigating whether specific patient subgroups within the ESRD population on maintenance hemodialysis, such as women and ethnic minorities, may benefit from revised hemodialysis regimens.

Another consideration for improving hemodialysis outcomes is the health status of persons as they enter dialysis. Although the population on hemodialysis is predominantly older and suffering from type 2 diabetes, children can develop serious kidney disease that leads to ESRD. Because their bodies are still developing, children are especially vulnerable to the complications imposed by ESRD and dialysis. In a recent study, researchers found that children requiring dialysis have a poor clinical outcome if they also have experienced poor growth. NIDDK-supported researchers prospectively monitored a national cohort of over 2,300 children initiating dialysis in terms of school attendance, hospitalization rate, and survival. The results showed that poor growth preceding the initiation of dialysis was associated with poor school attendance (an important marker of functional status for children), increased frequency of hospitalization, and a two-fold greater risk of death as compared with more normal growth. The researchers emphasized in their report that aggressive nutritional measures should be taken to maximize growth in children as soon as chronically deficient kidney function is documented.

Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, Allon M, Bailey J, Delmez JA, Depner TA, Dwyer JT, Levey AS, Levin NW, Milford E, Ornt DB, Rocco MV, Schulman G, Schwab SJ, Teehan BP, and Toto R: Effect of dialysis dose and membrane flux in maintenance hemodialysis. *N Engl J Med* 347: 2010-19, 2002.

Furth SL, Stablein D, Fine RN, Powe NR and Fivush BA: Adverse clinical outcomes associated with short stature at dialysis initiation: A report of the North American Pediatric Renal Transplant Cooperative Study. *Pediatrics* 109: 909-13, 2002.

GROWING PROSTATE-GLAND-LIKE STRUCTURES FROM STEM CELLS

One hope held by researchers working with stem cells is to one day be able to use them in treating injury and disease. Thus, scientists are working to isolate and characterize adult stem cells from all major organs and tissues, including those of the genitourinary tract, such as the prostate. NIDDK-supported investigators recently defined a small region of the mouse prostatic duct, called the "proximal region," that contains cells having stem cell properties. The cells divide slowly (slow cycling), have high proliferative potential, and have the ability to give rise to complex glandular structures *in vitro*.

Using established cell-labeling analytic techniques, the researchers initially localized slow cycling cells to the proximal prostatic duct. They then dissected proximal and distal duct cells from animals, grew them *in vitro*, and examined their proliferative capacity. Proximal prostatic duct cells were shown to generate over 200 times as many progeny as those originating from the distal prostatic duct, indicating that the proximal cells have high proliferative potential.

To examine whether the cells were capable of forming glands, the researchers grew the proximal and distal prostatic duct cells in a special collagen gel matrix. Cells from the proximal region gave rise to numerous, large-branched ducts that contained typical prostate cells that produced prostatic secretory substances. By contrast, cells from the distal duct region produced far smaller and simpler gland-like structures.

These results strongly suggest that the proximal region of the prostatic duct contains a concentration of stem cells that are capable of reconstituting large, branched glandular structures that produce prostatic secretory substances. Future characterization of these cells—particularly their developmental pathways, cell surface markers, and the signals involved—should help in understanding the processes involved in prostatic homeostasis and the causes(s) of prostate diseases, including benign prostatic hyperplasia (see "Vision Statement—John McConnell, M.D.") and prostate cancer. To help accelerate such studies, the NIDDK recently launched an initiative to encourage researchers to develop new, cell-selective tools and methods applicable to studies of the prostate, bladder, and other organs of the genitourinary tract.

Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D, Shapiro E, Lepor H, Sun TT and Wilson EL: Proximal location of mouse prostate epithelial stem cells: A model of prostatic homeostasis. *J Cell Biol* 157: 1257-65, 2002.

EXPANDING BASIC RESEARCH STUDIES OF THE URINARY BLADDER

Through research, scientists are discovering that the urinary bladder is much more than a reservoir for liquid waste. Rather, it is a dynamic organ with many important structural and physiological properties. It is made of smooth muscle connected to nerves and lined with a unique "transitional epithelium" that makes specialized proteins to protect the bladder cells from the urine. It is also the only organ in the body able to fill and then empty on command. Proper function of the bladder is vital to rid the body of wastes and prevent infections. Unfortunately, millions of Americans suffer from both acute and chronic bladder disorders that interfere with proper bladder function. Urinary

tract infections, congenital obstructions, and urinary incontinence are just a few of the common clinical conditions affecting the bladder and lower genitourinary tract. Researchers have turned their attention to understanding the normal biology of the bladder and its component tissues, in order to gain insights that may lead to better tests, treatments, and prevention strategies for bladder disease.

Unfolding Umbrella Cells Requires New Membrane: In a recent study, NIDDK-supported researchers found that, as the urinary bladder increases in volume with filling, changes are elicited in the cells that line the bladder to ensure that the permeability barrier of this organ is maintained. To simulate bladder expansion, researchers stretched animal bladder tissue in an experimental chamber under normal physiological conditions, and performed a series of rigorous electrical, physical, microscopic, and biological analyses. Important new basic information about bladder structure was revealed. One major finding concerned the cells lining the inner surface of the bladder, called "umbrella" cells. Previously, it was hypothesized that increased cellular membrane surface area was achieved by simple unfolding of existing membrane structure, like the unfolding of an umbrella. Instead, the researchers found that the cells actually increase in size by synthesizing new membrane.

Upon stretching, the umbrella cells also underwent a dramatic change in shape, decreasing in depth and increasing in length. Vesicles—small "bubbles" of membrane components—also fused with the cell membrane, a process known as exocytosis. Stretching also prompted a rapid and continual secretion of protein from within the umbrella cells into the external environment. Surprisingly, stretching also induced involution of the umbrella cell membrane into vesicles by an energy-dependent process called endocytosis; the vesicles were subsequently degraded within the cell. While exocytosis and endocytosis are known to occur simultaneously in all cells, even under resting conditions, it previously was thought that endocytosis in umbrella cells took place only as bladder volume decreased. The specific functions and regulations associated with these newly discovered structural changes in umbrella cell membranes will be the subject of future investigations.

Uncovering fundamental knowledge on urinary bladder structure and function through basic research is expected to lead to more effective treatments for bladder diseases and disorders. Expanding basic research studies of the bladder and lower genitourinary tract is one of the strategic priorities put forward by the Bladder Research Progress Review Group (PRG). This task force was convened by the NIDDK two years ago to assess and make recommendations about needs and future directions in bladder research (see sidebar, "The Bladder Research Progress Review Group: Generating a Strategic Plan for Bladder Research"). The Bladder Research PRG released its report in August 2002. Already, guided by recommendations contained within the report, the NIDDK expects to launch two new initiatives later in 2003 that are intended to support bladder studies. One initiative will encourage basic research on the biology of the bladder. The other initiative, entitled "Basic Research Related to Interstitial Cystitis," will support research on the underlying causes of interstitial cystitis (IC), a painful and debilitating bladder disease (see "Patient Profile—Kara Fishbein Goldman"). It is anticipated that new knowledge gained from this research will feed into the development of new therapeutics for bladder diseases.

Truschel ST, Wang E, Ruiz WG, Leung S-M, Rojas R, Lavelle J, Zeidel M, Stoffer D, and Apodaca G: Stretch-regulated exocytosis/endocytosis in bladder umbrella cells. *Mol Biol Cell* 13: 830-46, 2002.

HEREDITARY HEMOCHROMATOSIS: A COMMON MUTATION THAT MAY NOT COMMONLY LEAD TO DISEASE

Hereditary hemochromatosis is a genetic disease that causes overabsorption of dietary iron. Because the body cannot excrete excess iron, it accumulates to toxic levels in body tissues ("iron overload") and leads to a number of complications, including diabetes, heart arrhythmias, and cirrhosis of the liver. Hereditary hemochromatosis is linked to mutations in the *HFE* gene, which was identified in 1996. Over 80 percent of hereditary hemochromatosis patients have a specific mutation in both copies of the *HFE* gene, a mutation called C282Y, which alters the *HFE* protein.

However, there are conflicting data about the actual risk of developing clinical symptoms when a person has two copies of the C282Y mutation. It had been proposed that being a C282Y "homozygote" may be necessary but not sufficient to cause clinical hereditary hemochromatosis. The C282Y mutation is highly common—one in 200 to 500 Americans are C282Y homozygotes. Because hemochromatosis is treatable with phlebotomy (periodic bloodletting), but its associated complications are not easily reversed, it is important to ascertain whether population screening for *HFE* mutations will effectively prevent disease.

To determine how well the genotype *HFE* C282Y predicts clinical disease, NIDDK-supported investigators screened 41,038 individuals to find those with the C282Y mutation. They then assessed whether individuals with the C282Y mutation were more likely to have symptoms or diseases associated with hereditary hemochromatosis than the control group, which lacked the mutation. At the same time, the researchers measured body iron stores. Iron burden was determined by two measures: saturation of the iron transporting protein, transferrin, and concentration of serum ferritin, an iron storage protein. By these measures, at least 75 percent of male and 40 percent of female C282Y homozygotes had significant iron burdens.

However, the prevalence of most of the clinical conditions associated with hereditary hemochromatosis, including diabetes, arrhythmias, and impotence, was not statistically different between C282Y homozygotes and the control patients. The only clinical symptoms occurring more frequently in C282Y homozygotes than in the control group was a history of hepatitis or other liver disorder, about a two-fold increase. Only one individual homozygous for C282Y had the broad spectrum of clinical symptoms diagnostic of hereditary hemochromatosis. From these results, the investigators estimate that less than one percent of C282Y homozygotes proceed to clinical disease.

Prevalence, intervention, and penetrance are the three most important factors in determining whether to engage in population screening for a genetic disease. The C282Y mutation in *HFE* is quite prevalent, and hereditary hemochromatosis is easily treatable. However, the results of this study suggest that the presence of the C282Y mutation alone is not an effective predictor of clinical disease. These findings will encourage investigators to seek secondary mutations or environmental factors—which may vary between populations—that influence the course of disease.

Beutler E, Felitti VJ, Koziol JA, Ho NJ, and Gelbart T: Pentrance of 845G→A(C282Y) HFE hereditary hemochromatosis mutation in the USA. *Lancet* 359: 211-18, 2002.

NEWLY DISCOVERED PROTEIN STABILIZES HEMOGLOBIN CHAIN

Hemoglobin in red blood cells carries oxygen throughout the body and is essential for life. Adult hemoglobin, or HbA, consists of two pairs of identical protein chains, two alpha-globin and two beta-globin chains. Red blood cells generally have balanced amounts of both chains that go into making the complete HbA molecules. However, if a red blood cell develops pools of excess "free" alpha- or beta-globin chains, these extra proteins can become unstable and clump

together, forming toxic precipitates that damage and ultimately kill the cell.

Beta thalassemia major, also known as Cooley's anemia, is a genetic disease caused by mutations affecting the beta-globin chain of HbA. These mutations reduce beta-globin production and create such an excess of alpha-globin that there is tremendous red blood cell death. This causes life-threatening anemia and other serious health problems. Cooley's anemia patients require lifelong blood transfusions to overcome the anemia and survive. Over time, these transfusions create health complications of their own, including transfusion-induced iron overload (see next section). For Cooley's anemia patients, iron overload and other complications require burdensome secondary therapies that can severely diminish quality of life. Scientists are therefore trying to develop molecular methods to control or compensate for the imbalances in hemoglobin production as an alternative therapeutic approach to Cooley's anemia and other forms of beta thalassemia.

In one recent study, an NIDDK-supported research team identified an abundant protein in red blood cells that interacts with and stabilizes free alpha-globin. Such stabilizing proteins are also called "chaperones." Studying both mouse and human red blood cells, the researchers found that Alpha Hemoglobin Stabilizing Protein, or AHSP, binds to alpha-globin, but not beta-globin or HbA. In biochemical assays and in live cells, recombinant AHSP prevented free alpha-globin from precipitating. Most significantly, when the team genetically-engineered mice to lack AHSP, the mice were viable, but their red blood cells showed evidence of globin precipitates, and red blood cell turnover was higher than normal—suggesting a physiologically important role for AHSP.

AHSP is the first red blood cell-specific molecular chaperone to be identified. These findings strongly suggest a role for AHSP in routine stabilization of free alpha-globin, and a possible role for AHSP in modifying disease severity in beta thalassemia. Future investigation of the AHSP protein may determine whether AHSP might be a possible therapeutic target for Cooley's anemia and other forms of beta thalassemia. Similar candidates may be identified through ongoing genomics studies that are using cutting-edge genetic and molecular techniques to identify human genes that modify disease severity in beta thalassemia, in hopes of finding possible therapeutic targets for this life-threatening illness.

Kihm AJ, Kong Y, Hong W, Russell JE, Rouda S, Adachi K, Simon MC, Blobel GA and Weiss MJ: An abundant erythroid protein that stabilizes free alpha-haemoglobin. *Nature* 417: 758-63, 2002.

WORKING TO LIFT THE BURDEN OF IRON OVERLOAD

Hereditary hemochromatosis and Cooley's anemia are quite different diseases, but share a common complication—iron overload. While iron is an essential nutrient used by all body cells, in excess of body needs it can accumulate to toxic levels in body tissues and cause a number of serious health conditions. The development and severity of these conditions, including organ damage, are closely correlated with the magnitude of the body iron excess. Patients with iron overload may suffer from liver disease with the eventual development of cirrhosis and, often, hepatocellular carcinoma, diabetes mellitus, gonadal insufficiency and other endocrine disorders, increased skin pigmentation, and iron-induced cardiomyopathy, which may be lethal. To effectively combat iron overload, both accurate techniques for measuring body iron stores and minimally burdensome therapies to remove excess iron are necessary. The NIDDK is supporting research in both of these areas.

Iron Overload Technologies: Physicians need to be able to assess body iron stores in order to provide appropriate care and treatment for their patients—such as phlebotomy for hereditary hemochromatosis patients and management of iron-removing "chelation therapy" for Cooley's anemia patients. Unfortunately, blood tests that measure iron saturation in iron transport and storage proteins

provide only a limited picture of total body iron stores. Because the most excess iron is stored in the liver, the "gold standard" for assessing body iron stores is a liver biopsy—an invasive, painful procedure. In April 2001, the NIDDK led an international workshop on the non-invasive measurement of iron to assess the current state of the science and to identify areas needing further investigation. The workshop participants concluded that additional research was needed to develop better quantitative means of measuring body storage iron that would be non-invasive, safe, accurate and readily available to improve the diagnosis and management of patients with iron overload.

The NIDDK has been at the forefront of developing new technologies for the non-invasive measurement of body iron. Thirty years ago, the NIDDK supported research that led to the development of the only non-invasive method for measurement of tissue iron stores that has been calibrated, validated, and used in clinical studies. The method employs a device called "SQUID" (superconducting quantum interference device). However, the complexity, cost, and technical demands of the instruments used in this method have restricted its widespread clinical use. With support from both the NIDDK and the National Heart, Lung, and Blood Institute, the researchers involved in the original development of SQUID are now engaged in a large-scale NIH Bioengineering Research Partnership project to modify the SQUID technology in ways to make it more affordable and ready for more widespread clinical use.

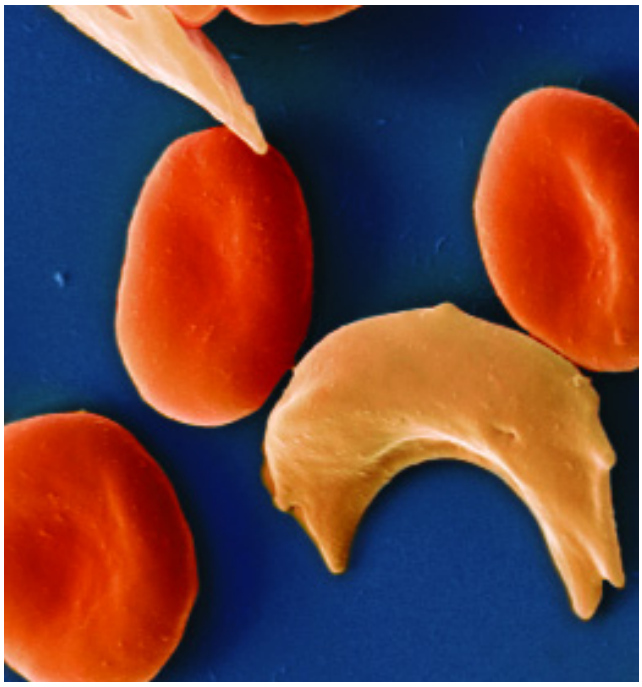
Magnetic resonance imaging (MRI) technology is already in widespread use for a number of clinical applications, from visualizing damaged tissues to monitoring chemical changes in the brain. At present, MRI also provides a means of probing the distribution of excess iron in the body, but further efforts are needed to make measurements quantitative. The NIDDK, in collaboration with the new National Institute of Biomedical Imaging and Bioengineering (NIBIB), has recently issued two new research solicitations to encourage both academic laboratories and small businesses to find ways to adapt MRI technology for application to the clinically useful measurement of body iron stores.

Alternatives to Current Chelation Therapy: Cooley's anemia patients with transfusion-induced iron overload must undergo "chelation therapy" to rid their bodies of excess iron. In chelation therapy, patients are infused by needle with a drug, deferoxamine, which will bind, or chelate, iron. The body can then eliminate these chelator-iron compounds. This treatment is the only therapy currently approved for use. However, the therapy is painful and takes up to 12 hours at a time, five to seven times a week. Since Cooley's anemia strikes in very early childhood, therapy can become an ordeal, and compliance can be a serious issue. Optimally, new chelator drugs would be less burdensome to use.

One drug that is progressing into clinical trials, HBED, appears to be more effective at chelation than deferoxamine, indicating that it may need to be used less frequently and for shorter periods of time. However, like deferoxamine, HBED still needs to be injected. The NIDDK is currently supporting basic and pre-clinical research studies to identify and evaluate oral iron chelator drugs as an alternative to injected chelators. Recently, a re-engineered version of a compound, desferrithiocin, was approved by the FDA for clinical studies. The Institute is also seeking proposals to perform pre-clinical evaluations of other iron chelating compounds.

ADVANCING THERAPIES FOR SICKLE CELL DISEASE

Sickle cell disease (SCD) is a genetic disorder affecting the essential oxygen-carrying molecule in the blood, hemoglobin. Like Cooley's anemia, SCD is caused by a mutation affecting the beta-globin subunit of hemoglobin HbA (see "Newly Discovered Protein Stabilizes Hemoglobin Chain"). However, whereas beta thalassemia patients lose production of the beta-globin molecule, SCD patients have defective beta-globin molecules.



Sickle cell disease causes the normally doughnut-shaped red cells in the blood to assume a crescent, or "sickle," shape (yellow cell). When large numbers of these sickle cells are trapped in tiny blood vessels, blood flow is blocked, causing tissue and organ damage and excruciating pain.
Photo: Dr. Mark Gladwin, NIDDK. ©2002 Nature Publishing Group (http://www.nature.com/).

Sickle cell disease results when an individual inherits a specific mutation in each of two copies of the gene encoding beta-globin. The mutant beta-globin chains are then incorporated into the complete hemoglobin molecules. The resulting defective hemoglobin molecules often stick together in long rods ("polymers") that cause the red blood cells, which are typically "doughnut"-shaped, to take on a "sickle" form. Such misshapen red blood cells are much more fragile than normal. Up to 10 percent of these cells die every day and spill their contents, which include large quantities of hemoglobin, into the bloodstream. In addition, sickle cells have difficulty passing through narrow blood vessels, and can block blood flow to vital organs or joints. This process causes extremely painful episodes, called "crises," that can last from a few hours to several weeks and may ultimately result in severe tissue damage.

Sickle cell disease is especially prevalent in African Americans. Current therapies for SCD are limited to drugs to help manage pain, treatment of complications, and experimental therapies to induce red blood cells to use alternate forms of hemoglobin instead of HbA. The only cure for severe cases of SCD is a bone marrow transplant, a serious operation with a number of risks. Scientists have recently made significant advances in pre-clinical and clinical research that should improve current therapies for sickle cell disease.

Gene Therapy for Sickle Cell Disease: In a recent pre-clinical study, genetic manipulation of beta-globin corrected sickle cell disease in two mouse models of the disease. From earlier studies, researchers knew that substituting "anti-sickling globins" for some of the mutant beta-globin chains could prevent the formation of hemoglobin polymers. In applying this knowledge toward the treatment of SCD, a significant challenge has been to incorporate a minimum therapeutic amount of anti-sickling globin into the beta-globin pool of almost every red blood cell. NIDDK-supported researchers designed a viral vector optimized for the transfer of genes into hematopoietic stem cells (the cells that give rise to all blood cells, found primarily in the bone marrow) and subsequent expression in red blood cells. Using the vector, they transferred an anti-sickling version of human beta-globin into bone marrow extracted from mouse models of SCD. This marrow was used to reconstitute the blood cells of both normal and SCD adult mice. The red blood cells that developed in these mice achieved a sufficient level of the anti-sickling beta-globin–up to 52 percent of total beta-globin–to significantly reverse the sickling of cells, enlargement of spleen, and disruption of urine concentration typical of SCD over the several-month course of the study. This study provides important "proof-of-principle" that gene therapy for SCD may someday be applied in treating human disease.

Stem Cells from Blood—A Possible New Approach to Therapy: Currently, the only successful treatment for severe SCD is a bone marrow transplant. As noted previously, bone marrow contains the undifferentiated stem cells that can develop into red blood cells that can replenish the patient's blood with healthy cells. Siblings are most likely to be eligible bone marrow donors. Siblings of sickle cell disease patients, however, have at least a 50 percent chance of having sickle cell trait—the inheritance of one copy of the sickle cell mutant gene. Sickle cell trait usually does not cause symptoms, but can increase a person's chances for infection or other complications arising from the bone marrow transplant procedure.

NIDDK researchers recently tested the safety and feasibility of isolating stem cells from the blood of individuals with sickle cell trait for transplanting into siblings with sickle cell disease. If successful, this would be an easier technique for collecting cells and could present less risk to the donors. Participants in the study were given a "mobilizing" agent to increase the number of their circulating stem cells before blood was collected. Researchers obtained sufficient numbers of stem cells from these individuals to predict that transplantation of mobilized stem cells into sickle cell disease patients could become a successful alternative to a bone marrow transplant from a sibling. Moreover, no serious adverse side effects were observed in the sickle cell trait participants. This research opens up an important new avenue of potential treatment for sickle cell disease.

Uncovering a Role for Nitric Oxide in Sickle Cell Disease Pain: The molecular mechanisms underlying sickle cell pain, and methods to reverse these episodes, have not been well understood. In a recent study, NIDDK researchers uncovered a significant clue about sickle cell pain. Nitric oxide (NO), a gas produced by endothelial cells lining the inside of blood vessels, has many functions throughout the body. For example, NO contributes to the regulation of blood pressure by promoting blood vessel dilation—an increase in vessel diameter. NO levels are closely balanced between production by endothelial cells and destruction by a hemoglobin "scavenging" system that converts NO into a biologically inactive form. The researchers hypothesized that abnormally high levels of free hemoglobin in the blood of sickle cell disease patients might cause too much NO to be inactivated by the scavenging system. The resulting loss of NO activity could lead to narrowing of blood vessels, which in turn could trap sickled cells and unleash a pain crisis.

The researchers found that, indeed, plasma from sickle cell disease patients had higher levels of hemoglobin and scavenged three times as much NO as plasma from normal volunteers. Removal of hemoglobin from sickle cell plasma reduced the NO scavenging to a level close to that of normal plasma. Furthermore, when sickle cell disease patients who were not undergoing a pain crisis during the study inhaled NO gas, it caused the rapid conversion of circulating hemoglobin to a form that interacts with NO only weakly. Thus, this study supports a role for hemoglobin scavenging of NO in sickle cell pain through the constriction of blood vessels. Importantly, the results suggest a potential therapy for alleviating the pain by inhalation of NO gas to reduce plasma hemoglobin and restore the ability of NO to properly regulate blood vessel size. Uncovering the role of NO in sickle cell disease will help scientists to develop new and more effective therapies for this painful and debilitating disease.

Kang EM, Areman EM, David-Ocampo V, Fitzhugh C, Link ME, Read EJ, Leitman SF, Rodgers GP and Tisdale JF: Mobilization, collection, and processing of peripheral blood stem cells in individuals with sickle cell trait. *Blood* 99: 850-5, 2002.

Pawliuk R, Westerman KA, Fabry ME, Payen E, Tighe R, Bouhassira EE, Acharya SA, Ellis J, London IM, Eaves CJ, Humphries RK, Beuzard Y, Nagel RL and Leboulch P: Correction of sickle cell disease in transgenic mouse models by gene therapy. *Science* 294: 2368-71, 2001.

Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO, Schechter AN, and Gladwin MT. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat Med* 8(12):1383-9, 2002.

ONGOING AND NEWLY LAUNCHED NIDDK EFFORTS IN KIDNEY, UROLOGIC, AND HEMATOLOGIC DISEASES

The NIDDK continues to advance basic and clinical research in kidney, urologic, and hematologic diseases through numerous initiatives, clinical trials, and support for investigator-initiated research. In addition to the previously described efforts in understanding and preventing kidney disease progression, the NIDDK, in collaboration with the National Institute of Neurological Disorders and Stroke and the National Institute of Child Health and Human Development, is planning to initiate a new study of chronic kidney disease in pediatric patients. This study will evaluate outcomes similarly to the CRIC study, but with a special focus on neurological function and other aspects of development that appear to be adversely affected in children with chronic kidney disease, but which have not been comprehensively studied as of yet. The NIDDK is also conducting the Family Investigation of Nephropathy and Diabetes study, a multi-center consortium to investigate genetic susceptibility in diabetic kidney disease, the leading cause of irreversible kidney failure.

The strategic plan from the Bladder Research Progress Review Group will be an invaluable tool for developing and guiding future research in the bladder and lower genitourinary tract. A number of its recommendations are already being implemented. In addition to strengthening basic research on the bladder in general and on interstitial cystitis in particular, the Institute is planning to establish a second cycle of the Interstitial Cystitis Clinical Trials Group, which has been testing and evaluating a number of therapeutic interventions for the painful symptoms of interstitial cystitis.

The NIDDK is also capitalizing on and extending the clinical value of the Medical Therapy of Prostate Symptoms (MTOPS) trial. This major clinical trial recently demonstrated that two drugs commonly used to treat benign prostatic hyperplasia (BPH), finasteride and doxasozin, are significantly more effective at preventing symptomatic BPH incidence and progression when given in combination. Together, they reduced risk of progression of BPH by 67 percent, versus 39 percent with doxasozin alone and 34 percent with finasteride alone (see also "Vision Statement—John McConnell, M.D."). The Minimally Invasive Surgical Therapies (MIST) Treatment Consortium for BPH is designing trials to assess the safety and efficacy of new surgical treatments for BPH; the first trial will include evaluation of the combination of surgery with a drug regimen similar to that used in MTOPS. Furthermore, biological samples collected during the MTOPS trial will be used by the MTOPS Prostate Samples Analysis Consortium to discover and validate biologic markers or genetic susceptibility tests for detection, risk assessment, and disease assessment of BPH—which could generate important new clinical tools for treating or managing the disease. The NIDDK is also initiating the Complementary and Alternative Therapy for Benign Prostatic Hyperplasia study, a large clinical trial to examine the effects and efficacy of two commonly used alternate therapies for BPH (also called "phytotherapies"), saw palmetto and *Pygeum africanum*.

Finally, facilitating future research on blood cell development and blood disorders, the NIDDK has initiated a Consortium of Genome Anatomy Projects (GAPs), involving eminent groups of hematopoietic stem cell investigators. These stem cell GAPs are aimed at developing the necessary biological procedures and reagents for characterization of cells of the hematopoietic lineage and characterizing gene expression patterns in these cells using advanced technologies and bioinformatics techniques. These projects will interact closely with similar projects related to bone, liver, intestine, kidney, and pancreatic cell development, with the promise for novel approaches to the study of pathogenesis and treatment of human diseases.

VISION STATEMENT

John McConnell, M.D.

The Future of Urology: Translational Research Opportunities and Challenges

As a leading medical researcher specializing in prostate biology and medical therapy for prostate disease, Dr. John McConnell has had the opportunity to both steer and observe the transformation of the urology field over the past two decades. Currently Executive Vice President for Administration at the University of Texas Southwestern Medical Center, Dr. McConnell had been engaged in both basic and clinical research as a professor of urology and director of the Prostate Disease Center, also at the University of Texas Southwestern Medical Center. Most recently, Dr. McConnell served as the lead investigator for the Medical Therapy of Prostate Symptoms (MTOPS) clinical trial, which demonstrated the benefits and efficacy of a combination drug therapy approach for benign prostatic hyperplasia (BPH). He also served on the Executive Committee of the Bladder Research Progress Review Group established by NIDDK (see sidebar, "The Bladder Research Progress Review Group: Generating a Strategic Plan for Bladder Research"). In his presentation to the NIDDK National Advisory Council in September 2002, Dr. McConnell offered a vision of the future for urology research that relies upon influencing the paths it will take, not just predicting them.

Major Challenges in Benign Urologic Diseases

Urologic diseases originate in several parts of the genitourinary tract—primarily the bladder, prostate, and urethra—and strike people across the age spectrum. The causes vary widely, from bacterial infections to anatomic abnormalities. Although the majority of these diseases are termed "benign" because they are non-cancerous, their symptoms can range from the irritation of the urinary tract to the inability to urinate normally, resulting in incontinence or urinary retention and creating the potential for toxicity and bladder or kidney infection. Benign urologic diseases can also interfere with normal sexual function. Many urologic diseases, such as congenital urinary obstruction and urinary incontinence, are somewhat age-dependent. Others, such as stone disease and interstitial cystitis, strike throughout life. All can severely decrease quality of life.

Dr. John McConnell

Dr. McConnell pointed out a number of clinical advances in urology from the past 20 years that have significantly transformed the field, improving patient care and disease outcomes. These include tests for levels of prostate-specific antigen (PSA), which can indicate abnormal prostate growth, and the development of laparoscopic surgery procedures for the kidney and prostate, which are less traumatic for the body than standard open surgery.

Yet, major challenges remain in several areas of benign urologic diseases research. As outlined in the accompanying figure, these challenges include identifying new therapeutic targets for benign

prostate and bladder disease, the assessment of a man's baseline risk for benign prostate disease, and developing more non-invasive techniques for diagnosis of urologic disorders. Organ replacement—namely, bladder replacement—is a particularly crucial challenge for combating pediatric urological disease.

In order for urologists to meet these challenges and continue the trend of successful clinical accomplishments, Dr. McConnell stressed the crucial need for improved progress in current basic urology research that will supply the next generation of therapies and surgical interventions for benign urologic diseases. "There have been a fairly large number of things that have really completely transformed the practice of urology in the 20 years that I've been engaged in urologic practice. I am somewhat pessimistic, though, unless there are further basic science breakthroughs in our understanding of basic prostatic growth and other aspects of urologic disease, that this list (of advances) may not change significantly in the coming decade."

Alternate Visions of the Future

Using the disease benign prostatic hyperplasia (BPH) as an example, Dr. McConnell presented a specific illustration of past accomplishments contrasted with future possibilities. BPH originates in the prostate, a walnut sized gland found wrapped around the urethra at the base of the bladder in men. This gland produces fluid that is a component of semen. Because of the prostate's location, however, prostate disorders can also lead to urologic problems. In men over fifty, the most common prostate disorder is BPH, a non-cancerous growth in prostate size that interferes with normal urination by squeezing the urethra. Symptoms and their severity vary from person to person, but include increased frequency or urgency of urination, weak streams of urine, and urine leakage.

Identifying major challenges in urology research is the first step toward making significant clinical advances in the future. In his Council presentation, Dr. McConnell listed some of the most pressing research needs for the "wide range of disease processes that impact the function of the genitourinary tract.

To illustrate past accomplishments, Dr. McConnell first noted the tremendous wave of new clinical findings and treatment options for BPH over the past decade. World-wide adoption of a standard for symptom evaluation, the AUA Symptom Score, has enabled standardization in the evaluation of treatment efficacy, while a less-invasive surgical procedure for BPH, transurethral resection of the prostate (TURP), has become the "platinum standard" for surgical treatment. Most significantly, there has been a radical change from exclusive treatment of BPH with surgery fifteen years ago to predominant use of drug therapy now, saving many men from the discomfort and complications of invasive treatment.

However, he also noted that these treatments and changes were fueled by scientific findings from 20 to 30 years ago, and expressed concern that the current state of prostate biology research is not keeping pace with the need for more clinical advances like these.

VISION STATEMENT

He then offered two alternate "visions of the future"—one in which basic research in prostate biology stagnates such that new therapeutic targets are not forthcoming and one in which basic research flourishes, leading to fundamental breakthroughs in understanding regulation of prostate growth. In the former, pessimistic vision, fourth generation drugs and non-validated phytotherapy regimens would provide the only "new" therapeutics for BPH. In the latter, optimistic vision, truly novel therapeutic targets would emerge, and there would be more and better diagnostic tools to use with BPH. The challenge is to ensure that the more optimistic vision prevails—not only for BPH, but for all benign urologic diseases.

Research Goals in BPH

To help address this challenge, Dr. McConnell identified a number of research needs and priorities for basic and clinical research on the prostate and on the bladder. For the prostate in general and BPH specifically, these include identifying and establishing genetics and biomarkers for disease susceptibility and predictors/assessors of response to therapy in BPH; the identification of new therapeutic targets; understanding the regulation of prostate growth (a "very urgent need"), understanding the bladder's response to obstruction in aging; and assessment of phytotherapies.

As Dr. McConnell pointed out, data gathered during the recently completed MTOPS trial provide key examples of gaps in knowledge that need to be filled in both basic prostate biology and BPH. The MTOPs trial was a large randomized clinical trial of over 3,000 men with BPH comparing the efficacy of two drugs that are currently used to treat the disease. The two drugs, finasteride and doxazosin, target different aspects of BPH pathology. Finasteride stops growth of and sometimes even shrinks the prostate. Doxazosin, on the other hand, is a so-called "alpha blocker" that targets alpha adrenergic pathways in prostate smooth muscle, decreasing the tension around the bladder and urethra that can interfere with normal urination. The landmark MTOPs trial demonstrated that using the two drugs in combination is much more effective at reducing the risk of progression of BPH than using each drug singly. It also showed that administering doxazosin alone over time (up to 5 years) does not significantly reduce the risk of acute urinary retention, or the complete inability to urinate. This indicates that "the alpha adrenergic-mediated smooth muscle tone is not the only story when it comes to the development of problems with urethral resistance, which is why men go into urinary retention."

Furthermore, the use of finasteride and similar drugs to "androgen ablate" the prostate does not reduce prostate size more than 13 to 16 percent, because the cells that respond to this treatment do not constitute the majority of the cells in the overgrown prostate tissue. The non-responsive cells, stromal cells, take up significant volume, but their biology is not well understood. According to Dr. McConnell, "as of today, we have no understanding of how that population of cells can be targeted for therapeutic intervention, and I think if I had to pick a single high priority area of research it would be that."

Research Goals in Bladder

The bladder is a balloon-shaped organ made of smooth muscle with a protective inner lining of specialized epithelial cells and proteins. But rather than being a simple sack that fills with urine and empties when appropriate, it is actually quite a complex organ. The NIDDK-led Bladder Research Progress Review Group, of which Dr. McConnell was a member, recently issued a strategic plan that is meant to guide current and future endeavors in bladder research. Dr. McConnell referred to the strategic plan and its delineation of research goals (see sidebar, "The Bladder Research Progress Review Group: A Strategic Plan for Bladder Research"), but also offered a summary of research needs. These include understanding the normal developmental biology of the bladder and the role that innervation

plays in determining the activities of bladder smooth muscle. Understanding the activity of bladder smooth muscle is especially important in bladder disease because "the common final pathway…for bladder failure, whether it is related to congenital anomalies,…obstruction, or infection, is a switch in the smooth muscle phenotype that leads these normally contractile cells to express large amounts of extracellular matrix (molecules)"—an inappropriate, pathological phenotype.

Other bladder research areas needing attention include understanding the normal biology of the epithelial cells lining the inner bladder wall; the impact of obstruction, aging, and inflammatory conditions on sensory pathways in the bladder that influence urination and contribute to pain; the development of mouse models for bladder disease and development—accompanied by the development of techniques and expertise that can be more readily applied to studying bladder function in such a small animal; and comparative studies of bladder function between men and women over the age spectrum to tease out problems common to aging versus problems due to differences in male and female anatomy. Finally, a high priority research need that affects all of these areas is the need for refinement and validation of tools and techniques used to assess the function of the lower urinary tract in humans.

Other Research Needs and Opportunities

In closing remarks, Dr. McConnell briefly touched on the role of advanced imaging technologies in the detection of urological disorders, noting that many people "go into urology actually because they have an interest in imaging," but also want to continue with direct patient care. In exciting new work, advanced imaging techniques such as magnetic resonance (MR) spectroscopy and positron emission tomography (PET) are being applied to looking at metabolic function of the bladder, detecting the presence or absence of prostatic disease, and to a variety of other lower urinary tract disorders.

He re-emphasized the need for research on urological organ replacement or substitution—primarily the bladder—as the best means to successfully combat congenital abnormalities and other pediatric and adult urological conditions. Tissue engineering, genomics, and stem cell research are all areas that hold promise for enabling clinicians to move beyond the limitations of reconstructive surgical repairs currently used for these conditions. The Bladder Research Progress Review Group has made specific recommendations in these areas as part of its strategic plan for bladder research.

Finally, he brought up androgen replacement—specifically, the administration of testosterone to men. The growing use of testosterone by men needs to be more closely examined for its long-term impact on health, as well as its true value as a therapeutic approach for men with borderline serum testosterone values. According to Dr. McConnell, "This really needs to be addressed. I think it will shortly become a major health issue."

"Push Complex Systems in the Right Directions"

Ultimately, Dr. McConnell proposed that in trying to "steer the future to some degree," it is useful to view the current state of the urology field as a complex system. If the "starting conditions" in such a system are appropriately adjusted and tweaked, down the road, the most positive outcomes may be realized. Thus, the NIDDK and its advisors may best influence the future of urology research by assessing the state of research and research needs, setting goal(s), providing research tools and funding, guiding the complex system of research relationships and research training in urology to obtain maximum productivity, and—perhaps most importantly—providing leadership. In this way, the NIDDK can continue to meet the challenge of ensuring an optimistic future in urology research and its ensuing clinical benefits for Americans suffering from urological disorders.

The Bladder Research Progress Review Group: Generating a Strategic Plan for Bladder Research

Diseases and conditions affecting the bladder and associated structures of the lower urinary tract are a leading cause of urinary incontinence, pelvic pain, and kidney failure, and they often contribute to poor quality of life. It has been estimated that 35 million Americans of all ages suffer from bladder disease and most have chronic conditions. Bladder problems have been reported to cost Americans more than $16 billion per year in health-related expenses, and this estimate does not take into account the associated physical and emotional disabilities that are considered "unmentionable" by many men, women, and children.

Significant basic and clinical advances in bladder disorders have been emerging more slowly than in other fields with similar disease burdens, such as diabetes and chronic kidney disease. Responding to this research gap, the NIDDK formed the Bladder Research Progress Review Group (Bladder Research PRG) in early 2000. This independent group of advisors consisted of scientists and medical professionals prominent in clinical and basic research and professional and lay organizations related to the bladder. They were asked to evaluate the research portfolios of NIDDK and NIH, identify research opportunities, and define unmet needs in bladder research The ultimate objective of the Bladder Research PRG was to develop a national "strategic plan" for bladder research—a document outlining goals and recommendations for future research and its implementation, to be used by the NIDDK, the NIH, and other entities as a guide for future initiatives.

From early 2000 to July 2001, the members of the Bladder Research PRG held discussions and intensive meetings to examine all areas of bladder-related problems categorized by diseases and by organ or tissue. Fourteen subcommittees focused their attention on specific bladder-related problem areas, including diabetes, muscle, development, inflammation/infection/interstitial cystitis, and clinical trials. The discussions and assessments performed by the Bladder Research PRG culminated in the report released in August 2002, *Overcoming Bladder Disease: A Strategic Plan for Research.* (http://www.niddk.nih.gov/fund/other/brprg_book.pdf).

In the report, the Bladder Research PRG identified four crosscutting priority areas in which strategic planning in bladder research will make important contributions to basic scientific knowledge and to the improved management and potential prevention of bladder diseases and conditions:

- **New technology-driven basic and clinical research,** to integrate advanced biotechnologies, such as gene microarray technology, into bladder research as has been successfully done for other organ systems and diseases.

- **Focused research for basic systems and disease,** to both improve management of and prevent bladder disorders and their complications, and to learn more about basic and abnormal bladder biology.

- **Epidemiology, outcomes evaluation, prevention, and bioethics,** to ensure the effective translation of basic research advances into clinical research to improve the diagnosis, treatment, and management of bladder disease.

- **Research infrastructure,** to provide greater support for programs, manpower training and technology (including information technology) in the area of the bladder.

These crosscutting strategic priorities emerged from the coordinated review of three overarching research areas: the basic science of the lower urinary tract, common clinical conditions, and methodologies and technologies for future research. The report presents a comprehensive summary of these areas, with specific goals and recommendations for each. The strategic plan will thus be invaluable in the development of research initiatives and in shaping the future of bladder research as a whole. Already, the NIDDK has responded to recommendations of the Bladder Research PRG in its development of two new research solicitations, one for basic research on the biology of the bladder, the other for basic research studies related to interstitial cystitis.

The work of the Bladder Research PRG will play an important part in efforts to reduce morbidity and mortality among those suffering from bladder-related diseases and disorders. As noted by Dr. Linda Shortliffe in the Letter from the Chair that introduces the report, "Advances in biomedical science and technology have opened the door for rapid and significant advances in bladder research that will improve the diagnosis, management, and prevention of bladder problems for many Americans."

PATIENT PROFILE

Kara Fishbein Goldman

Interstitial Cystitis

From left, Kara Fishbein Goldman and her twin sister, Beth. Both sisters are living with the bladder disease interstitial cystitis.

In 1994, when she was barely 20 years old and a student at the University of Pennsylvania, Kara Fishbein Goldman was diagnosed with interstitial cystitis or IC. She did not have too many symptoms of IC at that time, she says, but she had vulvodynia, chronic pain in the vulva, a symptom often associated with IC.

While Goldman was under anesthesia during treatment for the vulvodynia, her doctor dilated the bladder with sterile saline solution and used a cystoscope to visualize the bladder wall. He found areas of inflammation and pinpoint bleeding called glomerulations, which are characteristic of IC. Eventually, Goldman developed other symptoms of the disease—urinary frequency, urgency, and a burning sensation in the bladder—symptoms that she has experienced constantly for the past eight years.

Goldman describes her pain as feeling as though she has a bad bladder infection—a burning type pain that gets worse as her bladder becomes full—but "antibiotics don't cure it and other medications provide little relief," she says. "I feel like I have to (urinate) all the time, and it gets worse when the bladder is full. The pain is constant, but the severity varies."

Symptoms of IC vary from patient to patient, but they generally cluster around an urgent need to urinate; frequent urination both day and night; reduced bladder capacity; and feelings of pressure, tenderness, and pain around the bladder, pelvis, and genital area that may increase as the bladder fills.

"With IC, everybody is different. The pain presents in different ways in different people," Goldman explains. "Some have sharp pain, some have a burning sensation all the time where the inside of your body feels like it's on fire." She believes IC is more like a syndrome, a collection of symptoms. "Some people have some symptoms from the syndrome. Some people have other symptoms from the syndrome." In fact, Goldman's identical twin, Beth Fishbein, who developed IC a year after she did, has had milder symptoms. Currently in medical school, Fishbein is stabilized on a low dose of medication.

In some ways, Goldman and her sister were lucky. Their IC was diagnosed in the early stages, obviating the need to search for a diagnosis, a frustrating journey most people with IC are forced to undertake because many physicians are unfamiliar with the condition or because symptoms are often confused with other illnesses such as urinary tract infections. Even after IC has been diagnosed, many patients still have to search for new or different treatments that may help with their particular symptoms. In the year after her diagnosis, Goldman instilled a weekly "cocktail" of medications prescribed by

KARA FISHBEIN GOLDMAN

her physician into her bladder through self-catheterization. The bladder instillation included a local anesthetic, an antibiotic, and a steroid to reduce inflammation. This regimen kept her symptoms under control for about a year, but then the "cocktail" ceased to be effective, Goldman says, so she stopped it. Her pain gradually got worse, and became difficult to manage in the year before her wedding. She tried numerous oral medications, including tricyclic antidepressants, drugs that relax the bladder muscle, block pain, and have some effect on the body's allergic response, which appears to be involved in IC. Other drugs prescribed for her condition include a drug to relieve nerve pain; a drug that appears to augment the bladder lining; several pain medications; and drugs to relieve the bladder spasms that often accompany and worsen urinary urgency, frequency, and pain. All of the medications helped for only a little while, Goldman says, and a neurostimulation device implanted in the epidural space of her sacrum did not provide any relief.

Interstitial cystitis has no cure and no universal treatment; therefore, physicians must try several treatments to relieve symptoms. People with IC have responded to various therapies, but even so, a particular treatment may work only temporarily. Flare-ups and remissions also occur.

"The (pain medicine) doesn't ever make my pain go away," she says, "It just makes me tired and sleepy and less aware of myself." This creates a dilemma. She either works in pain or takes off and sleeps. During the workday, Goldman forgoes the pain medicine, but when the pain is severe, she takes off from work, takes the medicine, and sleeps. She says she's lucky because some people wake up from the pain; she does not. "There's nothing much I can do to make myself feel better," she says. "I'm either working or sleeping. I don't want to sleep the rest of my life." Goldman has tried to explain her illness to her students, co-workers, and friends to help them understand why she cannot function as well as she would like. She has had varying degrees of success. "People don't understand chronic pain," she says. "I look absolutely fine, and people can't see that I'm in pain. I'm not limping and I don't look particularly bad." In October 2001 and last spring, Goldman's pain was so severe she had to take quite a number of days off from her teaching job. "That year was really bad. I missed a lot of days of school, and I'm taking a leave of absence (this school term). This illness has affected my life in a big way," Goldman says, adding that she and her husband, Steve, had hoped to have children by now, but can't because of all the medications she's on.

Goldman says she feels fortunate to have an extremely supportive and caring spouse and parents, who have been very helpful in her often frustrating search for therapy that will ease her symptoms. In 1997, after realizing that scientists knew very little about IC, Goldman's parents, Bob and Laurie Fishbein, established the Fishbein Family Interstitial Cystitis Research Foundation, which endows pilot studies of IC. Goldman also acknowledges great support from the Interstitial Cystitis Association, which has provided her with information about IC and treatment options and has put her in touch with other people with IC.

So far, Goldman's search for effective treatment and pain relief has taken her to six gynecologists, three urologists, and two pain specialists in two states. She hopes that her yearlong leave from teaching will lead to better health and perhaps to the fulfillment of a long-delayed goal. "I want to be free to go wherever I need to go in the country to see consultants," she says. "I'll take it easy and try to feel better. Maybe if I can get off the drugs, I'll try to get pregnant…. I'll revise as I go. I'll keep trying different things."

STORY OF DISCOVERY

Cardiovascular Disease and Kidney Disease: Teasing Out the Link

Are two diseases related or just coincident? Sometimes the answer comes through careful analyses of large sets of numbers—numbers of patients and their associated risk factors. In the case of kidney disease and cardiovascular disease, such epidemiologic studies have pointed to a connection between end-stage renal (kidney) disease and risk of cardiovascular disease. Now researchers are faced with teasing out answers to the question: why?

The United States Renal Data System (USRDS), established in 1987, is a national data system that collects, analyzes, and distributes information about end-stage renal disease in the U.S., and is supported by the NIDDK in conjunction with the Federal Centers for Medicare and Medicaid Services. End-stage renal disease (ESRD) is a state of irreversible kidney failure in which a person requires either dialysis or a kidney transplant in order to stay alive. The USRDS has collected comprehensive data on over 92 percent of Americans with ESRD and releases an Annual Data Report every year; researchers can then analyze these data to discover emerging trends in both causes of ESRD and causes of death in ESRD patients.

A connection between ESRD and death due to cardiovascular disease (CVD) in a small number of patients on hemodialysis was noted nearly three decades ago. However, it is the careful analysis of patient data available in the USRDS database that has enabled researchers to recognize the enormity of the connection between patients requiring dialysis and their subsequent deaths from CVD. According to the latest Annual Data Report (2002), CVD (primarily coronary artery disease, left ventricular hypertrophy, atherosclerotic heart disease, and congestive heart failure) is the leading cause of death in ESRD patients. Studies using recent USRDS data revealed that death rates from CVD in dialysis patients are 20 to 40 times higher than in the general population, and an extensive retrospective study showed that 73 percent of dialysis patients who suffer a heart attack die within two years.

The figures for mortality due to CVD in ESRD are striking—and ominous. Groups at highest risk for developing ESRD include the estimated 17 million Americans with diabetes, the elderly, and ethnic and racial minorities, as well as people with hypertension, genetic renal disease, or a family history of renal disease. Determining the underlying relationship between kidney disease and CVD is not simple, however. The population at risk for developing CVD—independent of ESRD—is very similar to the population at risk for ESRD. In fact, some of the risk factors for CVD are indistinguishable from those for ESRD. Furthermore, researchers recently reported a higher prevalence of many traditional CVD risk factors in ESRD patients than in the general population.

Studying the USRDS numbers, epidemiologists realized that rates of pre-existing CVD in people initiating dialysis are very high, approximately 40 percent. This led researchers to suspect that CVD is developing during pre-ESRD states. Before ESRD, there is a prolonged state of progressive loss of renal function, referred to as chronic kidney disease. The degree of chronic kidney disease is established by measuring how efficiently the kidneys can filter out toxins from the blood, known as the glomerular filtration rate. When glomerular filtration rate decreases, bloodstream levels of a number of waste products increase. Small studies recently indicated that, just as

in ESRD, there are higher rates of death from CVD in people with chronic kidney disease. A recently launched prospective study, the "Chronic Renal Insufficiency Cohort," will assess risk factors for both the progressive decline in kidney function and the development of CVD in a large study population with chronic kidney disease. Through careful data analysis, the scientists aim to determine whether chronic kidney disease causes CVD or is simply associated with it.

Although defining the link between chronic kidney disease and risk factors for developing CVD awaits the outcome of large prospective studies, researchers can still test ideas as to how factors traditionally associated with decreasing kidney function—uremia-related factors—might in fact also lead to CVD. Basic research studies have contributed significantly to a number of hypotheses about how observed uremia-related factors might increase the risk of developing CVD, including the following:

Uric Acid: Uric acid is a waste product of nitrogen metabolism. It is normally present in the bloodstream, where it is thought to act beneficially as an antioxidant. However, impaired renal function can lead to uric acid levels that are too high, causing health problems such as gout. Elevated blood levels of uric acid may also be associated with a greater risk of heart disease, although the epidemiologic data are still under debate. A recent study in rats suggests a possible mechanism for uric acid's proposed role in CVD, showing that elevation of uric acid increases blood pressure and causes kidney injury. The human gene encoding the uric acid transporter responsible for uric acid recovery from the kidney tubules was recently identified by researchers in Japan; now, its activity in chronic kidney disease and ESRD can be tested.

Salt-sensitive Hypertension: There is evidence in animal models that subtle renal injury, induced by local inflammation and vasoconstriction, may interfere with normal salt excretion from the kidneys. Sodium dysregulation in particular may, in turn, raise blood pressure and further damage the kidney, initiating a vicious cycle, resulting in permanent salt-sensitive hypertension that promotes CVD.

Homocysteine: Homocysteine is a modified form of the essential amino acid methionine. Normal blood levels of homocysteine are maintained primarily by the activities of folic acid, vitamin B12, and vitamin B6. Deficiency in these vitamins can lead to hyperhomocysteinemia (high levels of homocysteine)—as can decreased glomerular filtration rate. Mild to moderate hyperhomocysteinemia appears to contribute to CVD outcomes in both the general population and persons with ESRD. Although successful in the general population, B-vitamin supplementation is not effective in lowering homocysteine in ESRD patients; however, it does normalize homocysteine levels in both renal transplant patients and mild chronic kidney disease patients. A study called FAVORIT is now testing whether high-dose supplementation with folic acid, vitamin B12, and vitamin B6 will improve CVD outcomes in chronic kidney disease patients and stable renal transplant patients.

As scientists develop testable hypotheses about how chronic kidney disease might induce CVD, they will be able to shore up epidemiologic data with mechanistic data to explain any observed link between these two conditions, and move towards possible prevention and treatment of CVD induced by chronic kidney disease. Through these efforts, future analysis of data from the USRDS will hopefully become the more optimistic task of documenting a steady reduction in cardiovascular disease-related mortality in patients with end-stage renal disease.

The National Kidney Disease Education Program (NKDEP)

Early in 2002, the NIDDK launched the National Kidney Disease Education Program (NKDEP), whose mission is to raise awareness about the seriousness of kidney disease, the importance of testing, and the availability of treatment to slow or prevent kidney failure. An estimated 10 to 20 million Americans suffer from reduced kidney function, also called chronic kidney disease, and nearly 400,000 must have either dialysis or a kidney transplant to stay alive. The number of people developing kidney failure has doubled each decade for the last two decades, and disease statistics indicate that this trend is likely to continue. One of the most common causes of kidney disease is diabetes, and the rates of both diabetes and kidney disease are increasing simultaneously.

Fortunately, kidney failure can be slowed, if not prevented. Evidence demonstrates that good control of blood sugar and blood pressure can reduce the risk of developing kidney disease. Low protein diets can also consistently lessen kidney disease progression. In spite of these advances in treatment and prevention, only a small number of those who most need it are receiving proper screening or treatment. NKDEP's mission is to get information on prevention and treatment to those who can most benefit from it.

Racial and ethnic minorities suffer a far higher incidence and prevalence of kidney failure than Caucasians. Rates of kidney failure are disproportionately greater in African Americans, American Indian and Alaska Natives, Native Hawaiians and other Pacific Islanders, and Hispanic Americans. Diabetic kidney disease is the most common cause of kidney failure in all of the aforementioned minority groups except for African Americans. High blood pressure-induced kidney damage is the primary cause of kidney failure in African Americans, with diabetic kidney disease running a close second.

The NKDEP message is targeted at doctors and other primary healthcare providers, at people at high risk for kidney disease—especially those with diabetes, hypertension and/or a family history of kidney failure—and at insurers and others responsible for paying for healthcare. Currently in its first phase, the NKDEP is recruiting volunteers to conduct educational campaigns for at-risk African Americans and health care providers in four pilot sites. The message focuses on identifying risk factors for kidney disease, screening those at risk, and providing appropriate treatment for those who are diagnosed with kidney disease. The four pilot sites are Baltimore, MD; Atlanta, GA; Jackson, MS; and Cleveland, OH. After completing campaigns in these sites, the NKDEP will be able to identify and refine successful strategies and launch a broader national campaign. In its next phase, NKDEP will target its message to American Indians, Hispanics and Latinos. The ultimate goal of this educational campaign is to reduce complications and death due to kidney disease and kidney failure among all Americans.

VISION STATEMENT

Robert Schrier, M.D.

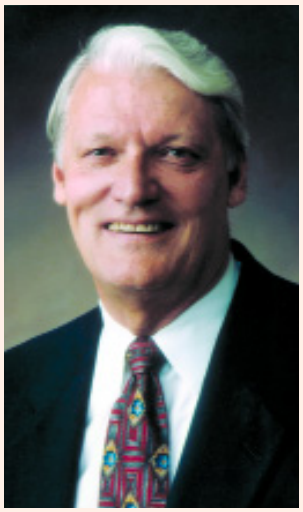
Kidney Disease Research: A Vision of the Future

Dr. Robert Schrier is Professor of Medicine at the University of Colorado School of Medicine. He has dedicated his research career to the study of both basic and clinical aspects of disease. Dr. Schrier has conducted extensive investigations of the molecular origins and causes of renal (kidney) failure and the mechanisms and pathways responsible for cell damage in this condition. His clinical research portfolio includes work examining the mechanisms responsible for kidney damage in people with diabetes, specifically on the role played by hypertension, the clinical term for high blood pressure. Additionally, Dr. Schrier is involved in a multi-institutional research project investigating hypertension in people with polycystic kidney disease, a particularly devastating genetic form of kidney disease. Dr. Schrier spoke at the September 2002 NIDDK National Advisory Council meeting about his vision of the future for kidney disease research.

Diabetes, Hypertension, and Kidney Disease

Type 2 diabetes affects about 8 percent of the U.S. population aged 18 and older and is strongly associated with obesity. Type 2 diabetes is also associated with aging, affecting 20 percent of Americans over 65 years of age. The current epidemic of obesity among Americans, coupled with the aging of the population, dramatically expands the number of people at risk of developing type 2 diabetes and its debilitating—and life-threatening—complications. This trend, if left unchecked, threatens to become a significant burden on the health care system in coming years.

Up to 65 percent of people with diabetes also have high blood pressure (hypertension), a condition that is associated with cardiovascular disease. Blood pressure is the force the blood exerts against the walls of arteries as it is pumped by the heart. High blood pressure is dangerous because it makes the heart work too hard and contributes to atherosclerosis (hardening of the arteries). It increases the risk of heart disease and stroke, as well as congestive heart failure, kidney disease, and blindness. Blood pressure is expressed as two values—the systolic pressure (as the heart beats) over the diastolic pressure (as the heart relaxes between beats). Blood pressure is measured in millimeters (mm) of mercury (abbreviated as its chemical symbol, Hg). "Normal" blood pressure is less than 135-140 mm Hg systolic and less than 85-90 mm Hg diastolic. "Optimal" blood pressure, which is associated with a reduced risk of heart disease, is less than 120 mm Hg systolic and less than 80 mm Hg diastolic. Blood pressure is usually presented as systolic/diastolic; for example, optimal blood pressure would be written 120/80 mm Hg and read as "120 over 80."



Dr. Robert Schrier

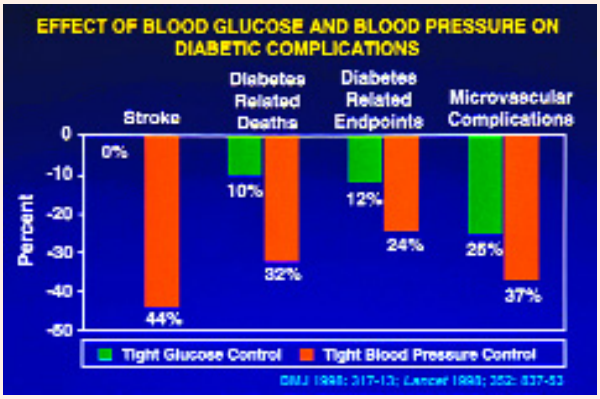
It is the vascular complications of diabetes that are responsible for the increased risk of heart disease, stroke, blindness, kidney failure, amputations, and premature death associated with the disease. Furthermore, both diabetes and hypertension are independent risk factors for cardiovascular disease, which is the leading cause of death in people with diabetes. Prolonged diabetes, in the presence or

absence of hypertension, is also associated with kidney disease, which can, if left unchecked, progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). Once kidney function diminishes to less than 10 to 15 percent of normal, ESRD patients require regular dialysis or a kidney transplant to survive.

Clinical Trials of Blood Pressure Control in Diabetes and Kidney Disease

The growing epidemic of obesity, coupled with the aging of the population, taken together with the toxic combination of diabetes and hypertension, bodes ill for the future. "There is an epidemic of (type 2) diabetes, not just in this country but in the world," noted Dr. Schrier. The leading cause of death among people with diabetes is cardiovascular disease. However, Dr. Schrier pointed out that several major clinical trials have shown that tight blood pressure control can be very effective in preventing cardiovascular complications in people with type 2 diabetes. The challenge now is translating these basic and clinical research findings into effective therapies, and defining and meeting the new challenges of the future.

Dr. Schrier reviewed a number of large-scale clinical trials that examined the consequences of lowering blood pressure levels in people with diabetes. Many of these trials compared the difference between lowering blood pressure to certain levels versus lowering it further. The United Kingdom Prospective Diabetes Study (UKPDS) found that tight blood pressure control (144/82mm Hg) was more effective in preventing vascular complications than either less tight blood pressure control (154/87 mm Hg) or tight control of blood glucose (sugar) levels. Reviewing the results, Dr. Schrier notes that tight blood pressure control had a more positive impact on "stroke, diabetes-related death, diabetes-related end points, and even microvascular (complications)" than tight blood sugar control.



Results from the United Kingdom Prospective Diabetes Study demonstrate a greater beneficial effect of tight blood pressure as compared to tight blood sugar control on diabetes complications.

Similarly, the Hypertension Optimal Treatment (HOT) trial found that aggressive control of blood pressure (less than or equal to 80 mm Hg diastolic) was particularly beneficial in patients with diabetes. The Appropriate Blood Pressure Control in Diabetes (ABCD) study showed that diabetes patients with hypertension had significantly higher rates of kidney disease than patients with normal blood pressure, and that drug therapy with an angiotensin-converting enzyme (ACE) inhibitor was more effective in reducing cardiovascular events than a calcium channel blocker. Similar results were seen in the Heart Outcomes Prevention Evaluation (HOPE) study. All of these trials indicate that tight blood pressure control in people with diabetes can significantly reduce the likelihood of cardiovascular complications and death. They also suggest that improved blood pressure control may be more important as blood sugar control in reducing complications and death in people with diabetes.

Several trials have focused specifically on the role of blood pressure control in the development of kidney disease. As Dr. Schrier noted, "Nearly 50 percent of all ESRD is due to diabetes, and (this value) continues to climb." In the hypertensive cohort of the ABCD trial, patients with diabetes and high blood pressure, but no signs or minimal signs of

kidney damage, maintained their starting level of kidney function over a 5-year follow-up period. In contrast, patients with overt diabetic nephropathy (kidney disease), in whom there was clear evidence of kidney damage, steadily lost kidney function over 5 years irrespective of whether their blood pressure was normally or tightly controlled. According to Dr. Schrier, this finding underscores the importance of identifying these patients early, when prevention measures can still be effective at halting degeneration. "You have to get there early, if we're talking about prevention (of ESRD)," he says. "Otherwise, you may be just delaying the need for transplant and dialysis for two or three years."

Polycyctic Kidney Disease and Blood Pressure

Polycystic kidney disease (PKD) is a genetic disorder characterized by the growth of numerous large, fluid-filled cysts in the kidneys. Over time, PKD cysts can slowly replace much of the mass of the kidneys, crowding and damaging the tissue and reducing kidney function, which can ultimately lead to ESRD. In the U.S., about 500,000 to 600,000 people have PKD and it is the fourth leading cause of kidney failure. There are two major, inherited forms of PKD: autosomal dominant PKD and autosomal recessive PKD. The autosomal dominant form accounts for nearly 90 percent of all PKD cases. Symptoms usually develop between the ages of 30 and 40, but they can begin earlier, even in childhood. About one-half of people with this form of the disease progress to ESRD. The autosomal recessive form of the disease is relatively rare, with symptoms beginning in the earliest months of life, even in the womb. "PKD is the fourth most common cause of ESRD," Dr. Schrier says, but "until 15 years ago, there was virtually no research on polycystic kidney disease." He adds, "Until the NIH focused on this disease."

As in patients with diabetes, people with PKD are particularly at risk for complications and progression to ESRD if they also have high blood pressure. In a study at the Heidelberg Outpatient Clinic, the percentage of patients with high blood pressure was significantly higher in those with the dominant form of PKD than in the general population, suggesting a correlation between PKD and hypertension. Furthermore, there is a correlation between the number and size of the cysts in the kidneys and the decline in renal function. PKD patients with hypertension progress to ESRD at a faster rate that those with normal blood pressure. Taken together, all of these factors add up to an ever-accelerating spiral, according to Dr. Schrier. "The larger the cysts, the larger the kidneys, the more hypertension, and the faster the progression to end-stage renal disease." Unfortunately, many patients with PKD are not treated until kidney damage has become irreversible.

But why do people with PKD have higher blood pressure to begin with? The answer to this question comes from insights into the molecular pathways cells use to "talk" to each other in the kidney. Researchers hypothesized that the ever-enlarging cysts in PKD stretch the blood vessels that line the cavities of the cysts. The compression of the blood vessels and resultant reduced blood flow result in damage to the kidney tissue. This damage activates a hormonal signaling system—the renin-angiotensin-aldosterone system—that has the end result of raising blood pressure. Studies led by Dr. Schrier have shown that this hormonal pathway is elevated in people with PKD, and that it correlates with the decline in kidney function over time, heart damage, and high blood pressure. Fortunately, aggressive control of blood pressure using ACE inhibitors (drugs that block the renin-angiotensin system) can reduce the progression of many of these complications.

However, recent clinical studies have suggested that "optimal" blood pressure (120/80 mm Hg) as opposed to blood pressure closer to normal values (in the range of 135 to 140 over 85 to 90) can have a dramatic effect on slowing the progression of patients with PKD to ESRD. "Focusing on research and education, we can do a better job controlling blood pressure," Dr. Schrier noted. The results of this recent focus have been dramatic: if one compares the period from 1985-1992 with the period from 1992-2001, the median age at which people with PKD progressed to ESRD rose from 53 to 63 years for men and from 57 to 61 years for women. This delay in the onset of ESRD means improvement in the quality of life of people with PKD. Dr. Schrier credited this improvement to better blood pressure control and the development of new drugs, such as ACE inhibitors, that make this control possible.

The Future of Basic and Clinical Kidney Research

As valuable as these clinical studies are, the importance of basic research—which lays the foundation of molecular knowledge upon which these trials stand—is also critically important. The NIH has an important role to play in both processes, Dr. Schrier says. "I think the NIH has had a major impact with respect to clinical studies and I would encourage the NIH to continue to do that."

Also of importance is the translation of these basic and clinical insights into changes in patient and physician behavior. To foster the translation of research advances into real-life improvements in the lives of patients, the NIDDK supports a number of education campaigns and information clearinghouses. The National Kidney and Urologic Diseases Information Clearinghouse increases knowledge and understanding about diseases of the kidneys and urologic system among people with these conditions and their families, health care professionals, and the general public. Pilot efforts of the National Kidney Disease Education Program aim to reduce morbidity and mortality from kidney disease by raising awareness about the seriousness of the problem and the importance of prevention, early diagnosis, and appropriate management (see sidebar, "The National Kidney Disease Education Program").

However, Dr. Schrier acknowledged that the expense of clinical trials—and translation of the results—can sometimes be an impediment to progress. In concluding remarks, he stressed the importance of building partnerships between the public and private sectors. "Given the cost of these trials and the industry interest in this area—with appropriate guidelines—this relationship can be supported and progress made," he said.

DR. WILLEM KOLFF AND DR. BELDING SCRIBNER

Two Pioneers in Dialysis Share the 2002 Albert Lasker Award for Clinical Medical Research

Willem J. Kolff, M.D., Ph.D., currently is Distinguished Professor Emeritus at the University of Utah School of Medicine in Salt Lake City. He previously was Head of the Division of Artificial Organs, and was Professor of Surgery, Research Professor of Engineering, and Director, Institute of Biomedical Engineering, at the University of Utah. Dr. Kolff invented the artificial kidney and dialysis technique in his native Holland before emigrating to the United States in 1950. In the course of his research on dialysis at the Cleveland Clinic in the early 1950s, he became involved in development of an artificial heart. Under his leadership in the late 1960s, the University of Utah's Division of Artificial Organs developed and tested an improved artificial kidney system that included the dialyzer and ancillary components. The major goal of the new design, developed under an NIAMD contract (see following text), was to produce an artificial kidney system that was efficient, easy, and safe to operate, and whose cost of manufacture was lower than the available systems. Under Dr. Kolff's leadership, the University of Utah developed as one of the world's leading artificial organ research centers. In 1982, under his supervision, the first fully artificial heart was implanted in a human patient, Dr. Barney Clark.

Belding Scribner, M.D., Professor Emeritus of Medicine at the University of Washington, pioneered the use of dialysis for patients suffering from kidney disease by inventing, in 1960, a shunt that could be hooked up to a dialysis machine. Also, through Dr. Scribner's investigations, the minimum level of adequate dialysis was established, lowering dialysis times previously standardized at 24-27 hours per week to 15-16.5 hours per week. In addition, Dr. Scribner established that dialysis schedules in the future would need to be individualized, taking into consideration clinical parameters such as body size, residual renal function, and dialysis frequency.

In 2002, Drs. Willem Kolff and Belding Scribner were the honored recipients of the Albert Lasker Award for Clinical Medical Research for their pioneering research on development of hemodialysis as treatment for acute and chronic kidney failure. Dr. Scribner was a grantee of National Institute of Arthritis and Metabolic Diseases (NIAMD), a forerunner to the present NIDDK, and Dr. Kolff was awarded numerous research and development contracts from the NIAMD.

When the NIDDK was established in 1950, medicine lacked any proven treatments to control end-stage renal disease (ESRD), and individuals with ESRD faced certain death. With the advent of effective kidney dialysis and transplantation, however, most chronically ill kidney patients no longer are confronted with such a dire prognosis. In fact, kidney dialysis alone has saved hundreds of thousands of lives.

Recipients of NIDDK awards have contributed significantly to the developments in kidney hemodialysis that have produced substantial benefits for ESRD patients. The earliest of these was Dr. Scribner's discovery in 1960 that a "no-stick" Teflon shunt—an artery-penetrating device that connects a vein to an artery—prevents the development of blood clots during dialysis. This was the first major breakthrough in hemodialysis. This simple but revolutionary idea provided the basis for regular and safer kidney dialysis, because without the threat of clotting a shunt could be left in place for multiple uses. Before this, patients received dialysis infrequently, because minor surgery was required before each treatment to re-

DR. WILLEM KOLFF AND DR. BELDING SCRIBNER

attach the necessary devices to veins and arteries. Although the shunt would later be replaced with the arteriovenous fistula* and synthetic graft, this reusable vascular access technique made it possible for the first time to keep patients with ESRD alive indefinitely.

Dr. Kolff reported the first successful dialysis to treat kidney failure in 1945. This was an extraordinary achievement and established the principle that cleansing the blood by passing it over a semi-permeable membrane could replace the function of failing kidneys. In its first applications, its use was limited to people who had temporary kidney failure, in part because punctures to remove and cleanse the blood irreparably damaged the vessels after fewer than a dozen times. Subsequently, with support from the NIAMD, Dr. Kolff and his colleagues designed a more-efficient, safer, and less expensive dialysis machine.

"Studies by Kolff and Scribner, funded by NIDDK, were pivotal in developing technologies that transformed dialysis from the realm of experimental possibility to effective and accepted clinical practice," said Josephine P. Briggs, M.D., Director of the NIDDK's Division of Kidney, Urologic, and Hematologic Diseases. "We are delighted that their critical contributions have been recognized."

Although kidney dialysis is an extremely successful therapy, it is of course no substitute for the healthy kidneys with which most individuals are born. For this reason, NIDDK scientists and grantees continue to explore the basic mechanisms of kidney function and disease with the aim of learning how to prevent ESRD. But, until the day that our understanding of abnormal kidney function dictates that no one ever need lose a kidney to disease, most of the millions of Americans who today suffer from irreversible kidney disease can rest assured that because of biomedical research, their disease does not mean certain death.

** a surgically-created communication between an artery and vein, usually performed in the forearm or leg of patients undergoing kidney dialysis.*

NIDDK Contributions to Dialysis

Dialysis as a practical treatment for kidney failure has evolved over centuries and continents. Many have played a role in developing this medical technology, starting with Thomas Graham of Glasgow, who first presented the principles of solute transport across a semipermeable membrane in 1854. Nearly 100 years later, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), originally called the National Institute of Arthritis and Metabolic Diseases (NIAMD), was established as part of the National Institutes of Health (NIH). Almost from the start, the NIDDK was committed to supporting research to combat kidney failure. Recently, investigators responsible for early advances in hemodialysis research were recognized with a major national award.

Willem J. Kolff, M.D., Ph.D., and Belding H. Scribner, M.D., shared the 2002 Albert Lasker Award for Clinical Medical Research for their pioneering work on chronic hemodialysis, a life-saving treatment for kidney failure. Their studies were funded in the late 1960s and early 1970s by the NIAMD.

Kolff reported the first successful dialysis to treat kidney failure in 1945. This was an extraordinary achievement and established the principle that cleansing the blood by passing it over a semi-permeable membrane could replace the function of failing kidneys. In its first applications, its use was limited to people who had temporary kidney failure, in part because punctures to remove and cleanse the blood irreparably damaged the vessels after fewer than a dozen times. But subsequent research by Kolff and Scribner led to a number of further advances that revolutionized the treatment, broadening its use to people who had permanent kidney failure. Funded by NIDDK, Kolff and his colleagues designed a more-efficient, safer, and less expensive dialysis machine, and Scribner and his team invented the first permanent device, made from Teflon, that allowed repeated, long-term access to the blood stream without piercing the vessels each time. Through his NIDDK-supported work on the diffusion of molecules through dialysis filters, Dr. Scribner also established minimum dialysis times and developed data needed to tailor treatment to the needs of individual patients.

The United States Renal Data System

Continuing its commitment to advance knowledge about ESRD, the NIDDK, in cooperation with the then Health Care Financing Administration, established the United State Renal Data System (USRDS) in 1987 to provide more extensive epidemiologic and demographic information for scientific purposes. The original mandate of the USRDS included four goals: (1) characterize the total population of renal patients and describe their distribution by sociodemographic variables across treatment modalities; (2) report on incidence, prevalence, and mortality rates and trends over time; (3) develop and analyze data on the effect of various treatment modalities by disease and patient group; and (4) identify problems and opportunities for more focused special studies of renal concerns. In 1992, two more goals were added: (5) conduct cost-effectiveness studies and other economic studies of ESRD and (6) support investigator-initiated projects to conduct biomedical and economic analyses of patients with ESRD. The USRDS has published an *Annual Data Report* every year since 1989 and has conducted a large series of special studies.

Beyond invaluable trend data, USRDS has had practical clinical implications, such as comparisons of outcomes of hemodialysis *vs.* peritoneal dialysis patients, peritonitis incidence by CAPD (chronic ambulatory peritoneal dialysis) connection technique, the role of dialysis efficiency on mortality risk in hemodialysis patients, and the role of histocompatibility antigen matching on kidney graft survival, to name only a few examples.

NIH Consensus Conference on the Morbidity and Mortality of Dialysis

In 1993, NIDDK sponsored a consensus conference on the morbidity and mortality of dialysis. Experts in general medicine, nephrology, pediatrics, biostatistics, and nutrition reviewed the available scientific data to develop a series of recommendations addressing several issues, specifically predialysis therapy, quality of life for patients with ESRD, quantitative evaluation of dialysis dose and adequacy, reasons for underdialyzing, cardiovascular complications, malnutrition, and research opportunities. The recommendations included a call for a minimum adequate dose of dialysis (a recommendation that would be repeated a few years later by the National Kidney Foundation's Dialysis Outcomes Quality Initiative) and clinical trials to explore whether a higher dialysis dose would result in even more favorable outcomes.

The HEMO Study

Following the consensus conference, the NIDDK initiated the multi-center HEMO clinical trial testing whether a higher hemodialysis dose or high-flux membranes or both would reduce mortality and morbidity, a hypothesis that evolved from Willem Kolff's work of nearly 60 years ago. The full-scale phase of the trial began in July 1994 with a data center and 15 participating clinical centers. The final results of this trial have just been reported; based upon the results, the investigators have concluded that these more intensive dialysis treatments do not, on average, provide any more benefit to patients as measured by patient survival.

The Hemodialysis Vascular Access Clinical Trials Consortium

The NIDDK has launched a new initiative that relates directly to Belding Scribner's early work on the Teflon shunt for vascular access. In 1998, NIDDK sponsored a workshop on Critical Issues in the Care of the Dialysis Patient. The workshop focused on nutrition and vascular access, which is often called the Achilles heel of hemodialysis because vascular access problems can lead to treatment failure. One recommendation that emerged from the workshop was to support basic investigations and clinical trials that explore ways to prolong the life of two types of vascular access ports, arteriovenous grafts and fistulas. In September 2000, NIDDK awarded grants to a consortium of institutions to conduct vascular access clinical trials. The consortium will conduct a series of multi-center, randomized, placebo-controlled clinical trials of drug therapies to reduce the failure and complication rate of arteriovenous grafts and fistulas in hemodialysis. Recently developed antithrombotic agents and drugs to inhibit cytokines will be rigorously evaluated in these large clinical trials.

Clinical Trial on Frequent Dialysis

A strong interest has developed throughout the renal community in the potential of intensified dialysis regimens, either slow nocturnal or short daily dialysis, to improve patient outcomes—a further refinement of Willem Kolff's original concept of improving the efficiency and effectiveness of dialysis treatment. Increasing dialysis frequency has a number of theoretical advantages as a strategy to improve dialysis dose, since clearance of accumulated toxins is greatest early in a dialysis run. In a small number of sites, with highly selected patient groups, markedly improved patient rehabilitation, better control of plasma phosphate and reduced erythropoietin requirements have been reported with more frequent dialysis.

In collaboration with the former Health Care Financing Administration, now the Centers for Medicare and Medicaid Services, the NIDDK organized an intensive two-day planning meeting in 2001 of dialysis experts to explore the feasibility of a randomized trial or observational studies of these new treatment strategies. Experts at the meeting were in general strongly supportive of the need for careful evaluation of these new therapeutic approaches. The feasibility of a randomized trial was discussed extensively; most meeting participants were strongly supportive of the need for the kind of rigorous evaluation only possible with randomized participants.

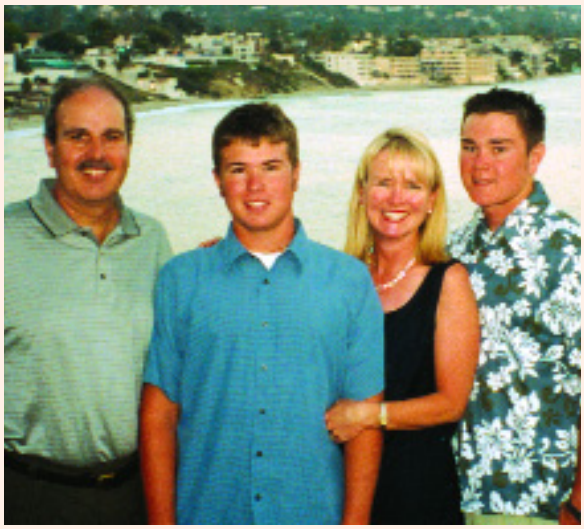
In December 2002, the NIDDK issued a Request for Applications (RFA) for cooperative agreement applications for a Data and Analysis Coordinating Center and two Coordinating Clinical Centers to design, develop and implement clinical treatment trials of frequent hemodialysis for patients with ESRD. The centers will propose trial designs for the studies. It is anticipated that two trials will be initiated, one comparing short daily hemodialysis with conventional dialysis and one comparing long nocturnal dialysis with conventional dialysis. The goal of the RFA is to test the feasibility of randomizing a representative sample of dialysis patients into either (a) conventional three times per week dialysis, or (b) slow nocturnal or short daily dialysis, and to obtain preliminary data on the impact of these modalities on patient well-being. It is expected that patients will be followed for a minimum of six months and that intermediate outcomes will be tracked, such as anemia, nutritional status, blood pressure, left ventricular hypertrophy, exercise tolerance, medication use, and hospitalizations. Based on the results of these trials, NIDDK will determine the advisability of continuing with a large scale trial of daily dialysis, powered to measure the impact of more frequent dialysis on hard endpoints, such as mortality and/or cardiovascular outcomes.

Advances in the treatment of kidney failure have come from many sectors, including private industry, educational institutions, and hospitals. The NIDDK has provided support and direction for much of the research that has led to incremental improvements and major breakthroughs in dialysis, and continues to look for ways to improve treatment and enhance quality of life for people with chronic kidney failure.

PATIENT PROFILE

Timothy Hawkins

Vesicoureteral Reflux



The Hawkins family (Tim, second from left). Tim has undergone two major surgeries to treat vesicoureteral reflux. Describing life since his surgery as "fairly normal," Tim is now attending college in California.

At the age of 18, Timothy Hawkins doesn't remember much about the pain and discomfort he experienced as a little boy, when he was frequently sick. His mother, Mary Pat, says he suppresses that memory because "he went through a lot." What Tim does remember is as follows: Going to the bathroom and seeing "all these lumps of things in my urine." Having high fevers and going in to the doctor's office and hospital for numerous tests—"ultrasounds and a VCUG" or voiding cystourethrogram, a diagnostic test that involves threading a catheter through the urethra, filling the bladder to maximum capacity with a radiographic dye solution, and following the flow of urine on x-ray as the child voids. Taking antibiotics every day between the ages of three and five to prevent kidney infection and to preserve kidney function. Going into the hospital with his parents for surgery when he was five years old for the removal of his right kidney. And having bad dreams after surgery that he thinks were caused by "the pain killers I took."

"My right kidney was deteriorating because I had reflux," Tim says. "The urine backed up into both my kidneys, but the right one was worse. When I was in kindergarten the right kidney had to be removed." Vesicoureteral reflux is the abnormal flow of urine from the bladder back into the ureter(s) and kidney(s). It is the most common cause of kidney failure in children and is usually diagnosed when a urinary tract infection or UTI occurs.

About one-third of children with UTI have reflux, which can occur from primary or secondary causes. Primary reflux occurs during fetal development when the tunnel in the bladder wall where the ureter inserts fails to grow long enough, thereby preventing the valve that forms at that juncture from closing properly. This permits backflow of urine and bacteria into the ureter(s) and even the kidney(s). Secondary reflux occurs as the result of conditions such as posterior urethral valves which obstruct the urethra, or bladder infection that causes swelling.

Tim's mother has more vivid memories of those years. She describes Tim's fevers, which were also caused by ear infections, as "raging" and frequent, and remembers putting him in a tub of tepid water to bring them down. She remembers her son telling her at the age of three that "sometimes poop comes out from where I pee" and the horror she felt when she saw dark lumps in the bottom of the toilet bowl after he urinated standing up. She remembers the anger and frustration she felt toward Tim's pediatrician when he told her over the phone that she was probably over-reacting and that she should give her son cranberry juice.

TIMOTHY HAWKINS

"Neither Tim's pediatrician nor the emergency room physicians took a urinalysis to see if the problem was caused by a urinary tract infection, a sign of reflux," Mary Pat says "A urinalysis is now done routinely when a child has a high fever."

She also remembers her father, a physician, urging her to ask Tim's doctor to perform an ultrasound to visualize Tim's urinary tract, and she remembers making an urgent phone call to her sister-in-law, a radiologist, to ask her to find someone who would do this when Tim's physician said it was unnecessary.

Mary Pat and her husband Jim soon decided that they needed to consult a pediatric urologist at a major medical center. "She placed Tim on continuous antibiotic therapy to see if his kidneys would heal," Mary Pat explains. Antibiotic therapy usually corrects secondary reflux caused by infection, and it prevents further damage if the cause is primary, that is, genetic.

Many children also outgrow reflux. During a period of "watchful waiting" to see if this would occur, Tim was monitored at intervals with urinalyses, ultrasounds, and VCUGs. However, when he was five years old, it became obvious that the right kidney was not going to heal, and it had to be removed.

By age nine, Tim's urologist determined that his reflux was primary and that it had not improved in the left ureter. Re-implantation of the ureter was now necessary because his urologist feared that in time it would cause further damage to his remaining kidney. The surgery involved lengthening the canal in the bladder wall where the left ureter inserts and then re-implanting the ureter.

Tim describes his life since his surgery as "fairly normal." He graduated from high school last June and is currently enrolled at Santa Clara University in California. Although he has not been able to play contact sports since his first surgery because of the risk of injury to his remaining kidney, he has found other outlets for his interest in sports. He played Little League baseball for a few years, learning to bat left handed so that if he were hit by a wild pitch, the side that doesn't have a kidney would absorb the impact. But when the pitches got faster, he decided it was time to drop out. In high school, he took up golf, which he has grown to love, and has played on his school team. "I sometime wish I could play football, though, but that's not an option," he adds.

Tim has been monitored with ultrasounds periodically to see if his remaining kidney is growing. "It grows larger to make up for the kidney that was removed," he explains. He recently had an MRI to check on scarring in that kidney. "Everyone has a little scarring. It can occur when you hold back going to the bathroom," he says. "But I'm more susceptible to it because of the reflux."

Recently, one of Tim's younger cousins was discovered to have reflux after he developed bladder control problems and side pain. At the age of six, the cousin's ureter was reimplanted. Because of the familial implications, Tim has begun to think about what a genetic disease will mean for his children.

"My doctor says that some reflux is genetic and there's a good chance that my kids would have the same problem with reflux," he says. "Maybe by doing research, doctors could test my kids before they develop problems or even before they're born so that they don't develop scarring and high blood pressure or lose a kidney."

ACKNOWLEDGEMENTS

Printed February 2003
For Administrative Use

Research:

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

Writing and Production:

Patient Profiles (except as noted below):
Larry Checco, Checco Communications

Patient Profiles:
(Kara Fishbein Goldman, Timothy Hawkins):
Carolyn Benson, Division of Kidney, Urologic, and Hematologic Diseases, NIDDK

Highlights of Research Advances and Opportunities:
Staff of the NIDDK Office of Scientific Program and Policy Analysis

Editorial Production:
Eleanor F. Hoff, Ph.D.,
NIDDK Office of Scientific Program and Policy Analysis

Design:
Gary Mosteller, Computercraft

Publication Coordination:
The Hill Group

For more information on the NIDDK and its mission, and links to other sites, visit:

http://www.niddk.nih.gov