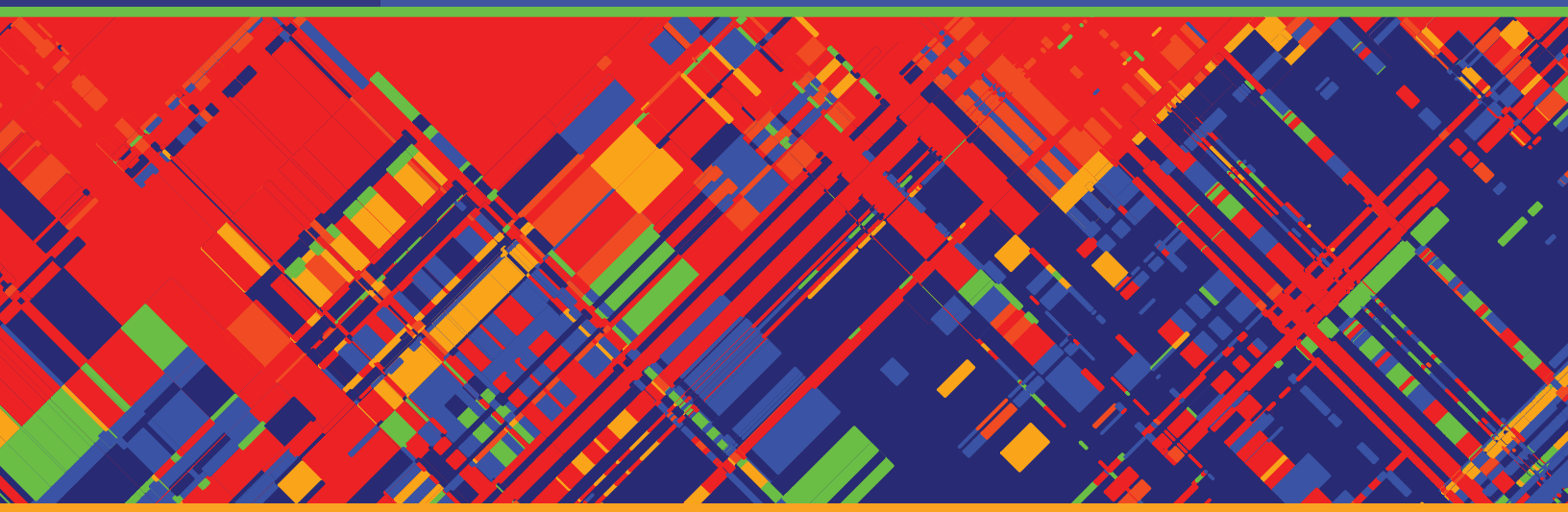
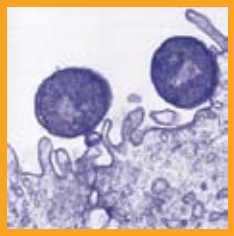
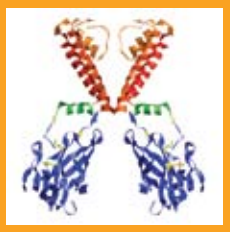
NIDDK

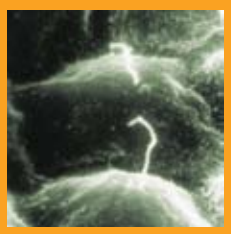
Recent Advances & Emerging Opportunities

January 2008









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

Images on the cover illustrate the wide range of research, from bench to bedside, supported by the NIDDK. The top, multi-color image shows results of a genome-wide association study of type 2 diabetes, with different colors indicating the degree to which some genetic sequences are associated with the disease. As described in this compendium, genome-wide association studies have led to the identification of genes involved not only in type 2 diabetes, but in other diseases within the NIDDK mission, such as inflammatory bowel diseases and type 1 diabetes. Inset images depict recent scientific discoveries made by NIDDK-supported researchers, which are described in this compendium. They also represent the people behind these and numerous other discoveries made each year. The NIDDK places a high priority on research training and mentoring, fostering the careers of new investigators, and supporting investigator-initiated research. Scientific progress is made possible by the contributions of talented scientists working in laboratories and clinics across the country. Likewise, the dedicated efforts of patient volunteers enable the clinical research supported by the NIDDK. The Institute is dedicated to fulfilling the promise of biomedical research—from supporting basic research and clinical trials to ensuring that research results are translated to the benefit of the public.

Genome-wide association study (top image): Image and permission provided by Drs. Michael Boehnke and Laura Scott. From Scott LJ, et al: Science 316: 1341-1345, 2007. Reprinted with permission from AAAS. Inset Images: Second image from left—Richard Nowitz for NIDDK; fourth image from left—Jupiter Images; for remaining inset images, see text at beginning of sections within this compendium for descriptions and credits.

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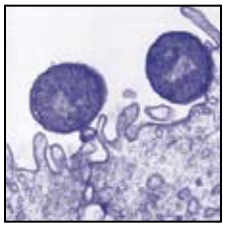

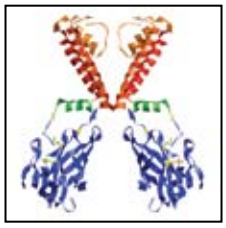

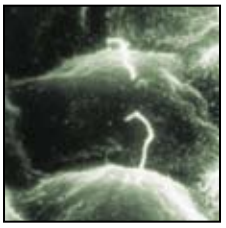

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CONTENTS

Message from the Director

As the new Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual compendium highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility, which includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases, such as cystic fibrosis; liver disease and other digestive diseases, such as inflammatory bowel diseases; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases, such as interstitial cystitis and prostate disease; and hematologic diseases, such as Cooley's anemia.

Now in its eighth year, this annual compendium illustrates recent NIDDK-supported scientific advances, such as the:

- Discovery of several new genes and chromosomal regions associated with Crohn's disease using genome-wide association studies.
- Identification of new genetic variants associated with type 1 and type 2 diabetes, also discovered through such studies.
- Demonstration that long-term cognitive function in people with type 1 diabetes is not impacted by recurrent episodes of low blood sugar.
- Finding that overweight or obese adults with type 2 diabetes who received an intensive lifestyle intervention for 1 year could lose a significant amount of weight, reduce their risk factors for heart disease, and reduce their medicine use.
- Revelation that bone plays a role in metabolism from studies in mice showing that bone secretes a hormone (osteocalcin) that contributes to obesity and diabetes.
- Discovery that some of the trillions of microbes living within the human gut may contribute to obesity.
- Demonstration of the ways in which *E. coli* and other bacterial strains interact with intestinal cells, which may contribute to human health or disease.
- Revelation that cilia—hair-like structures found on the surface of cells—play a role in the development of cystic kidney diseases and obesity.
- Finding that one surgical procedure is superior to another for treating urinary incontinence in women, based on the largest and most rigorous U.S. clinical trial undertaken on this topic.

This compendium also includes stories of patients who are directly benefiting from NIDDK-supported research. A new technology for monitoring blood sugar levels improved the lives of a teenager with type 1 diabetes and her family. A woman who participated in a gestational diabetes study learned of her increased risk for developing type 2 diabetes and has taken steps to remain diabetes-free. A man's quality of life has improved because of his participation in a clinical trial for benign prostatic hyperplasia. A participant in another clinical trial has received combination drug therapy that has kept his hepatitis B infection under control.

The NIDDK continues its efforts to ensure that knowledge gained from its major research advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's educational programs, such

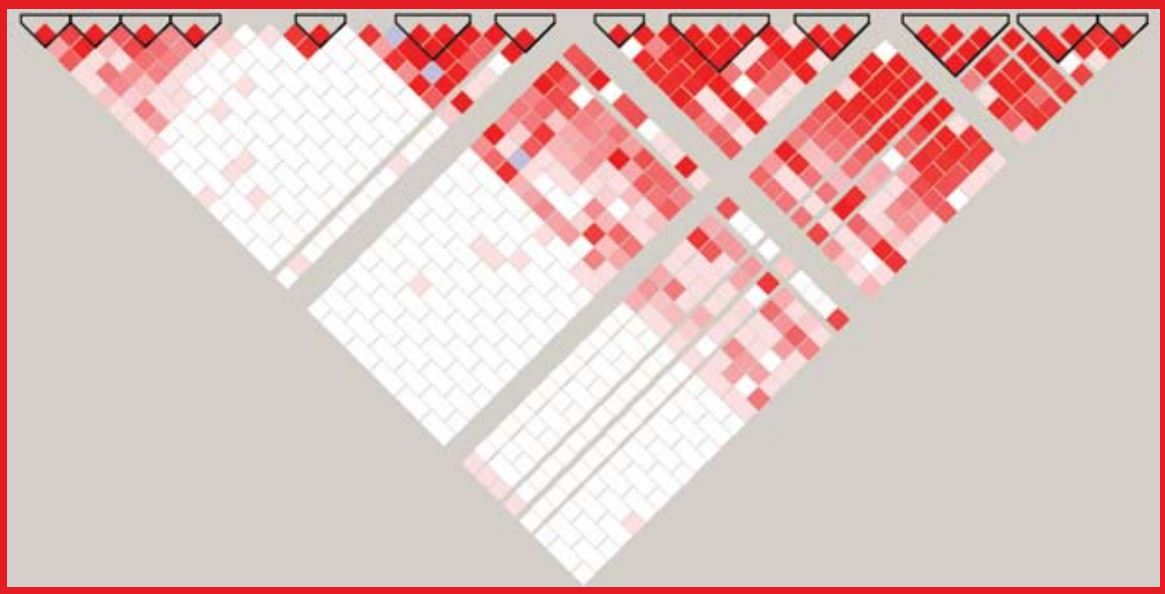
as the National Diabetes Education Program and National Kidney Disease Education Program, and NIDDK's co-sponsorship of the trans-NIH national education program for the prevention of childhood obesity entitled "We Can!" Significantly, NIDDK supports the following information and outreach programs: the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse. These programs distribute science-based information on diseases and disorders within the NIDDK mission. To make its informational resources more readily available to patients, healthcare providers, and scientists, the NIDDK also recently redesigned its website. I invite you to visit the new website at: *www.niddk.nih.gov*

The materials featured in this publication reflect the core mission of the NIDDK, including the Director's following guiding principles:

- Maintain a vigorous investigator-initiated research portfolio;
- Support pivotal clinical studies and trials;
- Preserve a stable pool of talented new investigators;
- Foster exceptional research training and mentoring opportunities; and
- Ensure knowledge dissemination through outreach and communications.

This compendium reflects only a fraction of the immense body of work performed by basic scientists, clinical researchers, and patient volunteers. We remain committed to translating their efforts into improvements in the health and quality of life of all people.

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



A genome-wide association study involves rapidly scanning markers across the genome to find genetic variations associated with a particular disease. Scientists conducting these studies analyze genetic differences between people with a particular disease and healthy people. As described in this document, recent genome-wide association studies have led to the identification of key genes involved in a variety of diseases within the NIDDK's mission. This image shows data from a genome-wide association study conducted to identify genes involved in inflammatory bowel disease. Understanding which genes contribute to disease not only enhances understanding of the underlying causes of disease, but could illuminate new targets for prevention and therapy.

Image provided by Dr. John Rioux and reprinted by permission from Macmillan Publishers Ltd: Nature Genetics, 39: 596-604, copyright 2007.

Cross-Cutting Science

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. Opportunities to make exciting new discoveries and advances are arising ever more rapidly with the development of new technologies, new approaches, and even new scientific disciplines. Described here are some recent studies of fundamental processes, ranging from the development of cells to the development of organisms, and new approaches and 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.

GENOME-WIDE ASSOCIATION STUDIES

A new era is beginning in the search for genes that contribute to disease. With the completion of the Human Genome Project and the International HapMap Project, scientists now have a set of research tools that make it possible to find genes that influence the likelihood of developing common diseases. Genome-wide association studies rely on these newly-available research tools and technologies to identify genetic differences between people with specific illnesses and healthy individuals. Through this comparison, it becomes possible to identify even subtle genetic differences between affected and unaffected people. This approach allows scientists to study diseases that involve many different genes, such as diabetes and inflammatory bowel disease.

To perform a genome-wide association study, scientists rapidly scan the genomes of many people for markers, called single nucleotide polymorphisms (SNPs). SNPs are single variations in the sequence of nucleotides or letters in the DNA code (A, T, G, and C). These variations occur in all people and most often are not associated with disease. To find SNPs that are associated with a particular disease, researchers compare many thousands of SNPs among people with and without disease, and identify candidate SNPs that are found significantly more or less often in one of the two groups. Such disease-associated SNPs can serve as strong pointers to regions of the genome where genetic risk factors reside. The first variants detected often do not directly influence disease susceptibility; however, the actual causal variant may lie nearby. This means researchers often need to take additional steps, such as sequencing every DNA base pair in that particular region of the genome, to identify the exact genetic variant that affects disease risk.

As described elsewhere in this edition of *NIDDK Recent Advances and Emerging Opportunities*, genome-wide association studies have already led to novel discoveries about genes involved in diseases within the NIDDK mission. For example, researchers have identified four new genetic variants associated with increased risk of type 2 diabetes and confirmed the existence of another six variants. This research brings to 10 the total number of genetic variants confidently linked to risk for the disease. Two of the newly-identified variations are in genes or regions not previously known to play a role in biological processes involved in diabetes. Their discovery opens new avenues of research for treatment or prevention of the disease. In addition, genome-wide association studies have led to the discovery of a new gene associated with type 1 diabetes. The gene is primarily expressed

in cells of the immune system and therefore may play a role in the immune system's attack of insulin-producing beta cells leading to type 1 diabetes onset.

Also described in this compendium are recent research advances from the NIDDK's Inflammatory Bowel Disease Genetics Consortium. Using genome-wide association studies, the Consortium identified several new genes and chromosomal regions associated with Crohn's disease. For example, the interleukin-23 (IL-23) receptor is a major susceptibility gene; the gene variant actually protects people against the development of Crohn's disease. They also identified *ATG16L1*, which is involved in a process (autophagy) used to degrade damaged cellular components and to help eliminate some pathogenic bacteria. The communities of bacteria that normally reside in the gut have been associated with Crohn's disease, so it will be interesting to see what role the new gene may play.

The NIDDK continues to support other genome-wide association studies. For example, ongoing studies are using samples collected from type 1 diabetes patients and their family members in the Epidemiology of Diabetes Interventions and Complications (EDIC) study and the Genetics of Kidneys in Diabetes (GoKinD) study. Scientists are using these well-defined collections to search for genetic variations associated with the development of long-term diabetes complications. Thus, the NIDDK is building on past investments in research and taking advantage of new and emerging technologies to pursue novel directions and gain new scientific information.

Why is it important to find genes that contribute to disease? Once new genetic associations are identified, scientists can use the information to learn more about the underlying cause of disease. For example, a gene could encode a protein that is involved in a signaling cascade with numerous other proteins. This knowledge could illuminate several new therapeutic targets for disease prevention or treatment. Furthermore, newly-identified genetic associations could be completely unexpected, as was the case for some type 2 diabetes genes. These types of surprising findings lead to brand new avenues for research that would likely not have been pursued otherwise. Therefore, even though the identification of new disease-associated genes is exciting in and of itself, the research sets the stage for even more scientific breakthroughs.

GENES AND THE ENVIRONMENT

New genomics technologies enable scientists to address questions of enormous complexity and importance, and genetic data are a key component in identifying and characterizing factors that influence health. However, the role of the environment should not be overlooked. Research initiatives at both the NIH and NIDDK levels aim to illuminate the intertwined roles played by genetic predisposition and environmental influences in the development and progression of disease.

The NIDDK is participating in an effort led by the National Human Genome Research Institute called the "Genes, Environment and Health Initiative" or GEI. This effort represents collaboration between geneticists and environmental scientists. It will use innovative genomic tools as well as new instruments for measuring the environmental factors themselves—from diet and physical activity to stress and substance addiction—in order to begin sorting out how these different factors affect a person's risk for a number of health conditions. To identify the genetic risks, researchers will use the rapidly evolving technologies used in genome-wide association studies to focus on common conditions, such as heart disease, cancer, and diabetes. The environmental component will begin by developing new technologies that accurately measure personal exposures with small, wearable sensors that can be used to assess environmental agents. The final component of the research strategy is to determine whether the effect of genetic variants that increase disease risk is different in the presence of environmental exposures.

In addition to this NIH-wide effort, the NIDDK is also supporting studies that address the role of genetics and environment in diseases within its research mission. "The Environmental Determinants of Diabetes in the Young" study is designed to solve this equation for type 1 diabetes, in which one or more as-yet unidentified environmental triggers spark autoimmune destruction of the body's insulin-producing cells. The hope is that a vaccine or change of diet, for example, could one day prevent the disease in those at risk. The project may also provide key insights on environmental causes of celiac

disease, which has overlapping genetic susceptibility with type 1 diabetes.

In celiac disease, gluten—a major protein in wheat, rye, and barley—triggers an immune response that damages the small intestine and interferes with the absorption of nutrients. Microbes that live in the human gut represent a key part of our body's internal environment. Recent NIDDK-supported research has established that there is bidirectional induction of genes between the body and intestinal bacteria, influenced by other environmental factors, such as nutrients. Future NIDDK efforts seek to expand understanding of the genomes of the gut bacteria—the microbiome—and detail the microbes' impact on human health. As discussed below, the NIDDK is also involved in the Human Microbiome Project, a new initiative recently launched as part of the NIH Roadmap. This project is aimed at more fully characterizing the human microbiome, and seeks to generate resources that will enable analysis of the role of the microbiome in human health and disease.

The new NIDDK Metabolic Clinical Research Unit at the NIH Clinical Research Center will permit intramural and extramural scientists an unprecedented opportunity to take environmental, dietary, and metabolic snapshots of normal, overweight, or obese patients. The facility will be an excellent resource for studies aimed at improving our understanding of the gene-environment interaction as it affects metabolic health, as well as for answering other research questions pertinent to obesity and overweight.

Another effort to tie environmental variables to metabolic health outcomes is an initiative on the obese and diabetic intrauterine environment. This initiative seeks to shed light on long-term impact on children born to mothers who were obese and/or diabetic during their pregnancies. Together, these examples of NIDDK research into these interactions promise to greatly increase our understanding of this critical interface between genes and the environment.

NIH ROADMAP FOR MEDICAL RESEARCH: SECOND COHORT OF ROADMAP PROGRAMS

The NIH Roadmap for Medical Research is an "incubator space" for nascent programs that cut across the NIH in terms of relevance or complexity, and which no single Institute or Center can address completely. The NIH Roadmap is now entering a new phase of scientific endeavor. Following the development of the first generation of Roadmap initiatives from inception to transition out of the incubator space, a second cohort of Roadmap programs is now in development. The new initiatives are being developed under the auspices of the Office of Portfolio Analysis and Strategic Initiatives (OPASI), a trans-NIH coordination and planning structure recently created within the NIH Office of the Director. While the new Roadmap initiatives are cross-cutting in nature, many of them intersect with NIDDK mission areas and could greatly aid research progress.

The second cohort of Roadmap initiatives began as ideas generated in Summer and Fall of 2006 by a wide range of stakeholders in the intramural and extramural scientific community, the patient advocacy community, and the general public. OPASI coordinated a review of the scientific concepts submitted and relevant NIH portfolios—applying criteria that potential Roadmap initiatives should meet. For example, Roadmap initiatives must be truly transforming; outcomes should synergize with missions of individual NIH Institutes and Centers to promote health; and trans-NIH participation must be required to address a scientific area in which no single NIH entity is likely to engage. Directors of the NIH Institutes and Centers then selected the areas, and the specific initiative concepts, that could be pursued. NIDDK representatives have participated in several of the Working Groups charged with developing these concepts.

Two concepts were approved as major Roadmap initiatives to be implemented immediately as 5-year programs: the Human Microbiome Project and the Epigenomics Program. The Human Microbiome Project aims to characterize the microbial content of sites throughout the human body and examine whether changes in the "microbiome" (including the collection of microbial species and their genetic material) are related to disease. The NIDDK Director serves as a co-leader of this Project, which will develop new tools and reference sequence data needed to study the human microbiome. Because the gastrointestinal (GI) tract is home to the body's largest collection of microbes, the Human Microbiome Project could greatly aid efforts to understand microbial effects on digestive health and disease.

The Epigenomics Program will develop resources for research in this area, which focuses on genetic modifications that change gene expression and function without altering the DNA sequence. This research will characterize the "epigenome" (a catalog of the stable epigenetic changes that occur in the genome) and its impact on health and disease. Plans include the creation of an international consortium, development of reference epigenomes, and establishment of a publicly available epigenetic database, as well as other research resources. In addition, support will be provided for fundamental discovery of novel epigenetic "marks"—marks on the chromosomes that activate or silence particular genes. Epigenetics may provide a mechanism by which environmental factors, such as diet, contribute to diseases such as type 2 diabetes and GI cancers. The NIDDK has therefore taken an active role in the Epigenomics Working Group, and serves as the lead Institute for the discovery initiative.

In the case of the two new Roadmap initiatives that will proceed to implementation, the release of Requests for Applications (RFAs) for 5-year programs began in Fall 2007, with awards to be made in Summer 2008 through Fiscal Year 2009. In addition to the immediate implementation of the Microbiome and Epigenetics Programs, other initiatives are being further developed for possible future implementation. These include a Phenotyping Services and Tools initiative, which was developed with leadership provided in part by the NIDDK Director. This initiative will develop a catalogue of human phenotypes for characterization of complex diseases. For the Protein Capture Tools/ Proteome Tools initiative, tools and resources will be developed to identify and isolate all proteins in the human body (the proteome). A pilot study will create a Genetic Connectivity Map—an effort to discover commonalities in gene expression patterns among diseases, responses to drug candidates, and genetic manipulations.

While these new Roadmap initiatives and pilot studies go forward, Roadmap Coordination Working Groups will continue to assess current efforts and future opportunities for cross-cutting collaborations in regenerative medicine, pharmacogenomics, and bioinformatics. The ongoing focus of the Roadmap Strategic Planning Working Groups will be on research training and research career development, health disparities, and effective administrative approaches to fostering scientific research.

More information on the second cohort of Roadmap programs can be found at:
http://nihroadmap.nih.gov/roadmap15update.asp

STEM CELLS, PROGENITOR CELLS, AND DISEASE APPROACHES

Stem cells have the potential to develop into many different cell types in the body. To better understand these cells and "progenitor cells," which have a more limited developmental potential, scientists continue to characterize their properties and seek potential new ways of using them to benefit patients.

Stem Cell Studies Provide New Insight into Aging: Clues about diseases associated with the aging process are beginning to emerge from studies of stem cells produced in the bone marrow. New investigations are helping close the gap in knowledge about these stem cells, including aspects of their microenvironment and factors that influence them in their primitive state or that control their activation and differentiation. For example, researchers have wondered why stem cells in the bone marrow of mice become less able to divide and replenish the supply of blood cells as they age. One theory proposed that, over time, these stem cells develop genetic mutations—but this idea has not been widely endorsed because these cells rarely divide. Hence, they are not thought to be susceptible to DNA damage, which is believed to be responsible for most mutations during cell division—mutations that may accumulate and give rise to cancer. To determine whether these stem cells accumulate genetic damage as they age, researchers recently isolated some cells from the bone marrow of normal young and old mice and indirectly determined the extent of their DNA damage. The cells from young mice showed no damage, whereas those from older animals showed extensive damage. In a different set of experiments, the researchers showed that mice with mutations in genes involved in DNA repair did not lose stem cells with age. However, the stem cells from these animals were much less effective in colonizing the depleted bone marrow of irradiated mice (which are similar to mice that have undergone a bone marrow transplant procedure) compared to

normal stem cells. This finding is consistent with the hypothesis that accrual of DNA damage may contribute to the diminished capacity of older animals to maintain health following stress or injury. These results could help to explain the development of leukemia and immune disorders that occur as people age.

Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, and Weissman IL: Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447: 725-729, 2007.

Potential New Stem Cell Approach to Transplantation: In another study, researchers developed a potential new approach to using stem cells for transplantation. Genetically matched embryonic stem (ES) cells are a potential source of cells and tissues for transplantation. Ideally, cells for transplantation will be well-matched with the recipient's cells in terms of proteins expressed on the cell surface, in order to minimize or prevent a response by the host's immune system. Most, if not all, ES cells are isolated from fertilized embryos. In an attempt to improve the degree of tissue matching, scientists isolated ES cells from an unfertilized mouse egg through a process called parthenogenesis, thus omitting the sperm's genetic contribution. The scientists identified stem cell lines retaining the identical "self" genetic information of the mouse egg donor and transplanted the cells into their respective donor for up to three months. The transplanted cells gave rise to tissues that were not rejected by the immune system of the donor mouse. While this research is very preliminary, the technique could offer an alternative method for deriving tissue-matched human ES cells that would not require destruction of a fertilized embryo.

Kim K, Lerou P, Yabuuchi A, Lengerke C, Ng K, West J, Kirby A, Daly MJ, and Daley GQ: Histocompatible embryonic stem cells by parthenogenesis. Science 315: 482-486, 2007.

Liver Regeneration from Bone Marrow Stem Cells: Researchers have uncovered an important clue to the cellular origins of the regenerating liver—one that comes from outside the organ itself, in the bone marrow. The liver has a remarkable ability to return to its original size after injury, resection of diseased tissue, and/or transplantation with a piece of donor tissue. Improved understanding of the source of the cells involved in repopulating the liver can inform the use of future cell-based therapies, as well as enhance fundamental knowledge of the organ's developmental and regenerative processes. Past research identified stem cells residing within the adult liver, called oval cells, as an important source of cells for the regenerating liver. However, conflicting reports existed regarding whether these oval cells originated from inside or outside the liver—namely, in the bone marrow. NIDDK-supported scientists recently conducted a series of animal experiments that strengthened the case for bone marrow-derived cells under certain conditions. In these studies, rats were given a chemical to inhibit expansion of cells within the liver, so that any observed liver regeneration could be attributed to cells from outside the organ. The rats then received a bone marrow transplant from a "donor" rat and underwent a surgical or chemical procedure to trigger liver regeneration. The transplant donor differed from the recipient in both genetics and gender. The genetic difference enabled researchers to observe donor bone marrow cells that had migrated to the liver during regeneration and became oval cells, then differentiated into mature liver cells. By providing a way to count the number of X chromosomes present, the gender difference between donor and recipient allowed scientists to determine that the oval cells had directly matured into liver cells, rather than simply fused with mature liver cells. Based on these findings, the researchers concluded that, in conditions of severe liver injury and inhibition of cells inside the liver, bone marrow cells may play an important role in regenerating the liver. Together with other studies, this work adds to the evolving understanding of the complex, multiple pathways controlling liver regeneration. Further research on the cellular regeneration pathway between the bone marrow and the liver has the potential to lead to useful cell-based therapies for liver disease.

Oh SH, Witek RP, Bae SH, Zheng D, Jung Y, Piscaglia AC, and Petersen BE: Bone marrow-derived hepatic oval cells differentiate into hepatocytes in 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration. Gastroenterology 132: 1077-1087, 2007.

Intestinal Stem Cells Lacking Tumor Suppressor Promote Polyp Formation: In laboratory studies, researchers have delineated how normal stem cells transition to cancer stem cells that can initiate the

formation of tumors in the intestine. In humans, the lining of the digestive tract undergoes continuous and rapid renewal throughout life. This renewal is sustained by a population of stem cells located in small recessed areas called crypts. These cells are "multipotent," which means that they are capable of developing into more than one cell type of the body. Although it has been known that the development of numerous polyps in the intestine results primarily from an abnormal increase in the number of crypts, the underlying cause remained unknown. Intestinal polyps are generally benign growths (non-cancerous) but some polyps can progress to cancer. To gain new insights, researchers studied a conditional knock-out mouse model, in which the tumor suppressor gene known as *PTEN* was deleted in the intestine. They determined that the deficiency in PTEN results in both an increase in number and an altered distribution of the stem cell population. The excess stem cells drive new crypt formation, which eventually results in formation of polyps. These findings offer opportunities to further characterize cancer stem cells—knowledge that will inform future cancer therapy studies.

He XC, Yin T, Grindley JC, Tian Q, Sato T, Tao WA, Dirisina R, Porter-Westpfahl KS, Hembree M, Johnson T, Wiedemann LM, Barrett TA, Hood L, Wu H, and Li L: PTEN-deficient intestinal stem cells initiate intestinal polyposis. Nat Genet 39: 189-198, 2007.

Colonic Stem Cell Markers and Regulatory Pathways: The recent identification of genes turned on specifically in regions of the colon containing stem cells has provided new insight into the molecular signals important for the healthy renewal of colonic cells. Cells of the human colon are renewed about once every week, as stem cells located in the bottom folds of the colon called "crypts" mature and migrate to the top of the colon surface, where differentiated cells perform key digestive functions. In order to understand the signals that differ between frank colonic cells and those that can develop into one of a number of cell types, researchers dissected the colon into a "top" (surface) and a "bottom" (crypts) section and looked for genes that were expressed exclusively in one area. When dividing the colon tissue this way, the researchers were able to include all the cells that play a supporting role in contributing signals to the stem cells and differentiated cells. As was expected, the differentiated top portion of the colon expressed genes that inhibit cell proliferation and genes that are required for digestive functions. Genes involved in cell proliferation and renewal were specifically turned on in the deeper, undifferentiated portion of the colon tissue. The contribution of several previously identified cell signaling pathways to the unique character of cells in the top versus bottom colon was examined as well. The researchers found that signaling through pathways involving molecules known as Wnt and Notch, which often regulate stem cell fate and early tissue development, was characteristic of the crypts of the colon. Bone morphogenetic protein (BMP) signals, typically activated during cell differentiation, were characteristic of the top portion of the colon. In contrast, BMP signaling was blocked in the crypt region by the presence of naturally occurring BMP antagonists produced by the supporting cells. The activity of these antagonists was found to activate Wnt signaling, inhibiting differentiation and promoting stem cell self-renewal and expansion in the crypts. These results extend to humans previous findings in mice, and elucidate the roles of different signaling pathways and molecules in creating an environment necessary for appropriate colon cell renewal. By further understanding the biology behind cell renewal in the colon, scientists may be able to develop therapeutics to stimulate or block these signals as needed when they go awry, such as in colon cancer.

Kosinski C, Li VSW, Chan ASY, Zhang J, Ho C, Tsui WY, Chan TL, Mifflin RC, Powell DW, Yuen ST, Leung SY, and Chen X: Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. Proc Natl Acad Sci USA 104: 15418-15423, 2007.

Identification of Gastric Stem Cells: Recently, researchers have discovered a cell population in the stomach with stem cell-like properties that may be involved in renewal of tissue, as well as tumor formation. To date, gastric stem cells have not been well-characterized due to a lack of specific cell markers to identify them within the tissue and to assist in their purification. In this study, researchers identified a marker to visualize these rare cells and to trace them to a specific region of the mouse stomach predicted to harbor stem cells. The areas suspected to contain these rare cells were the gastric glands, which are located at the base of pits on the interior surface of the stomach. These stem cells were able to proliferate and repopulate the entire gland with multiple cell types, indicating

that all the cells in the gland have the stem cell as their single source. Interestingly, expansion of these stem cells could be triggered by a pro-inflammatory molecule, suggesting that they could respond to injury within the stomach by replacing the injured cell types. However, the stem cells were also located in an area where gastric tumors commonly form, raising the possibility of their involvement in this process. These studies provide evidence for the importance of these newly recognized gastric stem cells in the maintenance and renewal of stomach tissue, and highlight their potential role in gastric cancer development. This initial characterization provides the experimental tools necessary for further studies to explore the properties of this unique stem cell population within the stomach.

Qiao XT, Ziel JW, McKimpson W, Madison BB, Todisco A, Merchant JL, Samuelson LC, and Gumucio DL: Prospective identification of a multilineage progenitor in murine stomach epithelium. Gastroenterology 133: 1989-1998, 2007.

Identification of Progenitor Cell Protein Involved in Pancreas Development: Scientists have identified a protein that is required for maintaining a pool of progenitor cells in the pancreas. Progenitor cells are cells destined to form a particular tissue or organ, such as the pancreas. As cells specialize, they often lose their ability to divide and make new cells. Progenitor cells that give rise to specialized cells generally maintain the ability to divide and can replenish lost specialized cells. Thus, research on progenitor cells is key to achieving the major goal of developing a means of growing unlimited quantities of insulin-producing beta cells in the laboratory. Such cells could be transplanted into people with type 1 diabetes to treat their disease. This goal is extremely challenging because it requires understanding the complex signaling pathways that are necessary to grow progenitor cells and induce them to become beta cells. Researchers made strides toward this goal with the discovery that a protein called SOX9 is necessary for maintenance and expansion of tissue specific precursors that give rise to the beta cell and other pancreatic cells. When they examined SOX9 expression in pancreatic cells during different stages of mouse embryonic development, the protein was found to be expressed in pancreatic progenitor cells, but not in more differentiated cell types. To examine SOX9's role in pancreatic development, mouse embryos were generated that lacked the protein in tissues that would normally form the pancreas. These mice did not develop normal pancreases, had extremely high blood sugar levels, and died within the first 4 days of life. Their rudimentary pancreases did not produce pancreatic hormones, such as insulin or glucagon. These results suggest that SOX9 is required for normal pancreas growth and cellular differentiation. Further studies indicated that SOX9 helps maintain the pancreatic progenitor cell pool by stimulating the cells' proliferation and survival. It is not yet clear whether SOX9 is involved in helping replenish stores of beta cells in adults, or is only involved during embryonic development. This research has enhanced understanding of pancreas development, which could inform the development of methods to grow insulin-producing beta cells in the laboratory for use in cell-based therapies for diabetes.

Seymour PA, Freude KK, Tran MN, Mayes EE, Jensen J, Kist R, Scherer G, and Sander M: SOX9 is required for maintenance of the pancreatic progenitor cell pool. Proc Natl Acad Sci USA 104: 1865-1870, 2007.

New Insights into Mouse Stem Cell Markers with Implications for Tissue Renewal: Therapies to regenerate diseased or injured organs will require an in-depth knowledge of how stem cells and progenitor cells develop into complex tissues and organs. For example, by developing methods specifically to recognize pancreatic progenitor cells, scientists will be better able to purify and grow these cells for transplantations to treat patients with diabetes or pancreatic disorders. In a recent study using a rodent model, researchers examined over 1,100 genes to determine which were expressed at key stages early in mouse pancreatic development, thereby potentially revealing genetic markers specific to pancreatic progenitor cells. Using this approach, the team found that the developing pancreas consists of five discrete domains of progenitor cells. They found and characterized one of these domains that was poised at the key position where progenitor cells branched into the three major functional cell types of the pancreas—hormone producing cells, including insulin-producing beta cells; digestive enzyme producing cells; and ductal cells that transport these digestive enzymes to the gut. Using the new markers they identified, the researchers were able to visualize the progression of these "multipotent" progenitor cells and their progeny within the mouse tissue into the

different pancreatic cell types. The ability to observe the changes in tissue location of these progenitor cells during development provided insight into how these cells physically populate the tissue of the pancreas. In a related effort to understand biological regulation of how a more general stem cell type—a mouse embryonic stem cell—develops into a broader array of tissues, scientists examined new and known stem cell markers to understand the relationship among these molecules. The researchers were able to create a map showing how the molecules influence and regulate one another. A thorough understanding of all stem cell markers will help scientists understand the natural development of tissues, and will be crucial for attempts to use these cells to repopulate injured or diseased tissues in humans.

Zhou Q, Law AC, Rajagopal J, Anderson WJ, Gray PA, and Melton DA: A multipotent progenitor domain guides pancreatic organogenesis. Dev Cell 13: 103-114, 2007.

Zhou Q, Chipperfield H, Melton DA, and Wong WH: A gene regulatory network in mouse embryonic stem cells. Proc Natl Acad Sci USA 104: 16438-16443, 2007.

Contrasting Developmental Programs in the Pancreas and Liver: Researchers have identified a fundamental difference in the way the pancreas forms during development compared to other organs, such as the liver. Working with mice, the scientists studied pancreatic progenitor cells. When the researchers selectively eliminated all the pancreatic progenitors from mouse embryos, each resulting mouse was born without a pancreas. When they eliminated some, but not all of the progenitors, the mice were born with smaller pancreases that never grew to normal size. In contrast, selective elimination of a large fraction of liver progenitor cells did not significantly affect the ultimate size of the mouse liver, presumably because the embryonic mice were able to rapidly compensate by creating more progenitor cells from a pool of pre-existing liver stem cells. These results imply that there are two types of organs—those whose size is determined by the number of progenitor cells that arise during development, and those whose size is controlled by different cues and resident stem cell populations. The experiments are also consistent with the observation that, while the adult human liver is capable of significant regeneration when damaged, the pancreas is much more limited in its ability to re-grow. From the standpoint of clinical research, the results indicate underlying developmental parameters that should be considered or circumvented in strategies for treating pancreatic damage resulting from injury or diseases such as diabetes.

Stanger BZ, Tanaka AJ, and Melton DA: Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. Nature 445: 886-891, 2007.

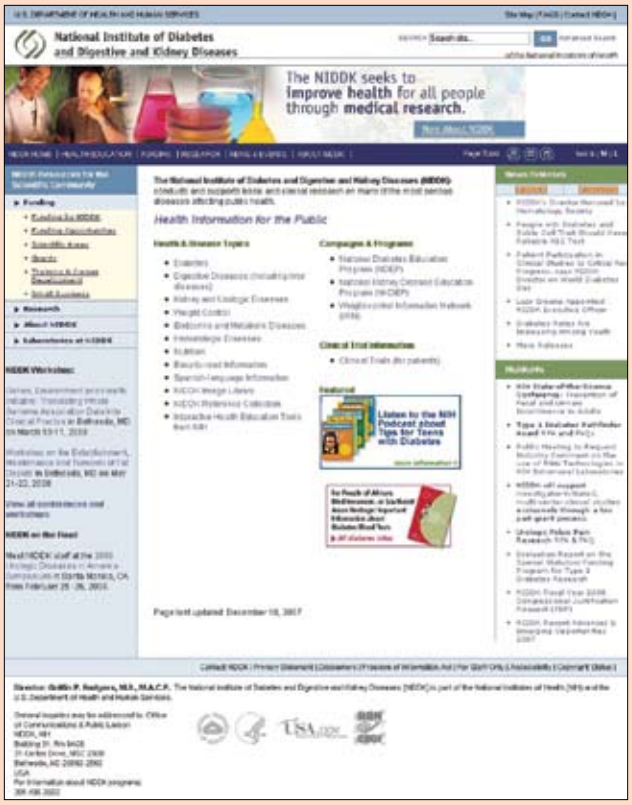
The New Public Face of NIDDK

In this "high-tech" era, more and more people use the Internet to access information. In particular, the NIDDK's website (*www.niddk.nih.gov*) receives nearly 2 million visitors each month. The NIDDK website is the public face of the Institute and is important for disseminating the many informational resources that the NIDDK has to offer.

In 2007, the NIDDK unveiled a new and improved website. The new design changed the look and feel of the website to be more appealing to visitors, but the high quality and fundamental architecture of information were preserved. Changes to the website include colorful graphics that help to convey NIDDK's science and public health mission, a new layout that is easier for visitors to navigate, and new features that highlight news and events.

The website still contains links to important health information for patients, families, and healthcare providers on diseases and disorders within the NIDDK mission. It also has information for scientists, such as information on research funding opportunities and research resources. Much information is tailored to specific groups of scientists, such as postdoctoral fellows and newly-independent investigators. The redesigned website makes it easier for these many different audiences to find the information that they are looking for in a timely and efficient way. In a press release announcing the launch of the new website, Dr. Griffin Rodgers, NIDDK Director, said: "Our new design should save researchers, health professionals, and the public valuable time finding important scientific and consumer health information. We are continually striving to make our resources more readily available to a wider audience and in the latest formats. The website plays a key role in helping to disseminate this information."

Please visit the new NIDDK website at *www.niddk.nih.gov*



The newly-designed NIDDK website: *www.niddk.nih.gov*

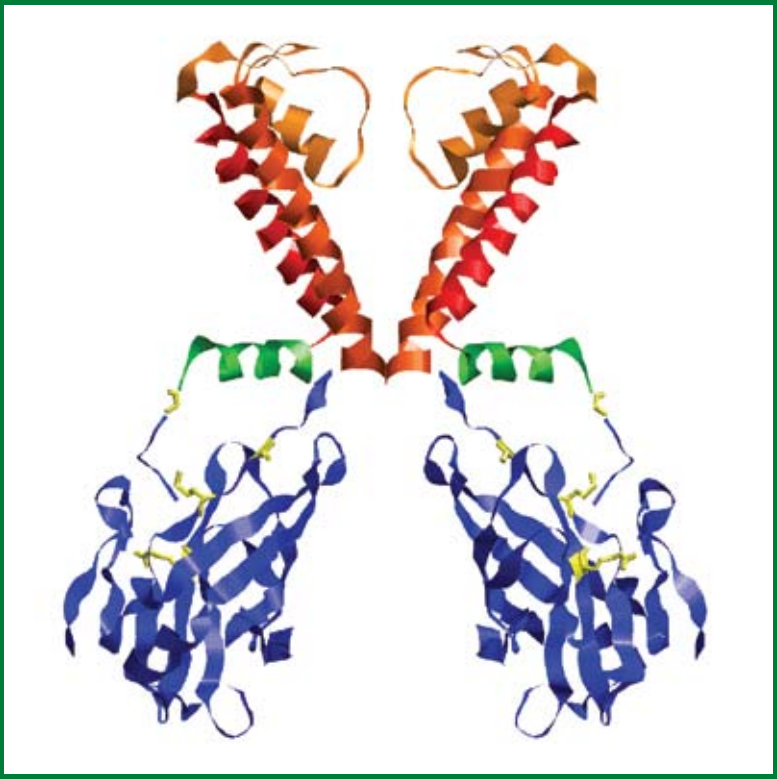


Illustration of two subunits of the Kir6.2 protein. This protein is part of a potassium ion channel that regulates the balance of potassium and calcium ions inside and outside of the beta cells of the pancreas, which in turn helps to regulate insulin secretion. As described in this chapter, mutations in the gene encoding this protein contribute to the development of permanent neonatal diabetes and type 2 diabetes. Mutations affecting the portions of the protein shown in yellow are associated with permanent neonatal diabetes.

Image provided by Dr. Andrew T. Hattersley. From Gloyn AL, et al: New Engl J Med 350: 1838 1849, 2004.

Diabetes, Endocrinology, and Metabolic Diseases

NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, they affect many millions of Americans and profoundly decrease their quality of life. Many of these diseases are complex—an interplay between genetic and environmental factors contributes to disease development.

Diabetes is a debilitating disease that affects an estimated 20.8 million people in the U.S.—over 7 percent of the total population—and is the sixth leading cause of death.[1] Diabetes lowers average life expectancy by up to 15 years,[2] increases cardiovascular disease risk two- to four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult onset blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2002—including costs of medical care, disability, and premature death—was $132 billion.[1] Effective therapy can prevent or delay diabetic complications, but approximately one-third of Americans with diabetes are undiagnosed.[1]

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone which is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin; and type 2 diabetes, in which the body becomes resistant to insulin signaling, with subsequent impaired insulin production.

Type 1 diabetes affects approximately 5 to 10 percent of individuals with diagnosed diabetes.[1] It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease, in which the immune system launches a misguided attack and destroys the beta cells of the pancreas. These beta cells, which are found within tiny cell clusters called islets, produce the hormone insulin. If left untreated, type 1 diabetes results in death from starvation despite high levels of glucose in the bloodstream. Thus, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels as well as they could if they had functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working on new beta cell replacement therapies meant to cure type 1 diabetes.

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

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

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[1] *www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf*

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

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

Gradually, the pancreatic beta cells secrete less and less insulin, and the timing of insulin secretion becomes abnormal. Treatment approaches for controlling glucose levels include diet, exercise, orally administered medications, and, in some cases, injected insulin. There are also an estimated 54 million adults in the U.S. who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[4] This population is at high risk of developing diabetes. Fortunately, the Diabetes Prevention Program (DPP) clinical trial has shown that patients with pre-diabetes can dramatically reduce their risk of developing full-blown diabetes with improvements in lifestyle or with drug treatment.

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

The NIDDK is supporting research to better understand the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the Institute's mission; such research will ultimately spur the design of potential new intervention strategies. In parallel, based on knowledge from past scientific research investments, the Institute is vigorously pursuing studies of prevention and treatment approaches for these diseases.

GENETICS OF DIABETES

Genes that Contribute to Developing Type 2 Diabetes and Genes that Affect Treatment Outcomes: Recent studies have dramatically increased knowledge about the complex genetic underpinnings of type 2 diabetes, in which a great many genes are each thought to play a small role in promoting or preventing the disease. Two NIDDK-supported studies took a genome-wide association approach (discussed in detail in the Cross-Cutting Science chapter) to help locate genes involved. Although the method does not generally identify the precise genetic alterations that cause these disease effects, it greatly narrows their likely location in the genome. The researchers thus newly identified at least four such type 2 diabetes-affecting genomic neighborhoods and confirmed several others that had been found previously, bringing the total known to about 10. With this information, scientists can begin to dissect the actual genetic changes that increase or decrease likelihood of type 2 diabetes, and thus provide potential new targets in the quest to prevent or treat the disease. A third study builds on previously reported genetic findings in just that way. Following a group of patients from the landmark Diabetes Prevention Program (DPP) clinical trial, researchers examined the impact of different versions of two closely linked type 2 diabetes genes both on disease progression and on the effectiveness of the lifestyle and pharmacologic interventions studied in the trial. Interestingly, one of these genes, which encodes the Kir6.2 protein, is also implicated in the rare genetic disorder neonatal diabetes, as described elsewhere in this chapter. The research indicated a genetic variant associated with increased diabetes risk did not alter progression to diabetes in people who already had pre-diabetes, suggesting the increased risk occurred earlier in the course of disease. Lifestyle changes were equally effective in preventing diabetes in those with different versions of the gene, but the drug metformin was less effective in preventing progression to diabetes in people with a particular genetic risk variant. Because metformin is one of the most widely prescribed medications for

[4] *http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm*

diabetes, it will be important to confirm this finding in those at risk for diabetes and to determine whether genetic variation affects response to metformin in those who already have diabetes. Taken together these studies illustrate the dramatic advances in the fight against diabetes that have become possible in the genomic era.

Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, and Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316: 1341-1345, 2007.

Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Boström K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Råstam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjögren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, and Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316: 1331-1336, 2007.

Florez JC, Jablonski KA, Kahn SE, Franks PW, Dabelea D, Hamman RF, Knowler WC, Nathan DM, and Altshuler D: Type 2 diabetes-associated missense polymorphisms KCNJ11 E23K and ABCC8 A1369S influence progression to diabetes and response to interventions in the Diabetes Prevention Program. Diabetes 56: 531-536, 2007.

New Gene Associated with Type 1 Diabetes Susceptibility: Scientists have discovered that variation in a region of DNA that includes the *KIAA0350* gene is associated with risk of developing type 1 diabetes. Using a genome-wide association approach, DNA from type 1 diabetes patients, unrelated controls, and family groupings including individuals with type 1 diabetes was examined to identify areas of variation potentially associated with the disease. In order to verify their results, the researchers conducted the genome-wide association study in two independent populations. Results from both populations indicate that three DNA variations, called single nucleotide polymorphisms (SNPs) in the region including the *KIAA0350* gene are associated with decreased risk of developing type 1 diabetes. With the approach used in this study, it is difficult to determine conclusively that *KIAA0350* is the gene that is influenced by these SNPs, and thus may confer protection from type 1 diabetes. However, *KIAA0350* is the only known gene in the area and the protein product of this gene is found primarily in cells that are part of the immune system. It is possible that this gene product could play a role in mediating immunity, which may have significant implications for the way the immune system reacts to the pancreatic beta cells that are eventually destroyed in type 1 diabetes. The discovery of the association of the *KIAA0350* region adds to the increasing understanding of the genetic basis of type 1 diabetes. Previously established genes associated with type 1 diabetes account for only half of the genetic risk of developing the disease; therefore, research into additional genetic markers is greatly needed. This discovery provides new avenues for exploration as researchers probe the function of this gene in the hope of establishing causes and developing new treatments for type 1 diabetes.

Hakonarson H, Grant SF, Bradfield JP, Marchand L, Kim CE, Glessner JT, Grabs R, Casalunovo T, Taback SP, Frackelton EC, Lawson ML, Robinson LJ, Skraban R, Lu Y, Chiavacci RM, Stanley CA, Kirsch SE, Rappaport EF, Orange JS, Monos DS, Devoto M, Qu HQ, and Polychronakos C: A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. Nature 448: 591-594, 2007.

TYPE 1 DIABETES RESEARCH

Recurrent Episodes of Low Blood Sugar Do Not Impact Long-Term Cognitive Function: Research has brought good news to people with type 1 diabetes: recurrent bouts of low blood sugar (hypoglycemia) do not affect long-term cognitive function. Low blood sugar is a serious and frightening

complication of type 1 diabetes. Because of its dangerous acute effects, it remains an obstacle to the practice of intensive glucose control that was proven to dramatically reduce the development of diabetic complications by the Diabetes Control and Complications Trial (DCCT) and subsequent studies.

To determine the effects recurrent episodes of low blood sugar may have on cognitive function, the DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group examined 1,144 participants from the original DCCT trial. The DCCT compared intensive management of blood glucose to conventional control in people with type 1 diabetes. At the outset of the DCCT, which began in 1983, participants also underwent a comprehensive battery of cognitive tests. When these tests were repeated 6.5 years later, no adverse effects were observed among either the intensive or control treatment groups. Although those results were promising, additional follow-up was needed to determine the long-term effects of hypoglycemic episodes. Therefore, the researchers repeated the cognitive analysis on participants after an average follow-up time of 18 years. In general, researchers found that measures of cognition did change over the nearly two decade follow-up, which is to be expected as people age: some measures tended to improve, while others declined. However, when the researchers stratified the cohort according to whether they were in the intensive or the control group, they did not see a difference in cognition. The same was true when they stratified according to number of hypoglycemic events: hypoglycemia did not cause cognitive decline. Only when the researchers stratified according to HbA1c (a measure of general blood glucose control) did they discern a statistically significant difference: better glucose control tended to translate to better motor skills. Even though acute episodes of hypoglycemia are worrisome, this research suggests that they do not result in long-term damage to patients' brains. The results also further support the recommendation that patients should practice intensive blood glucose control to prevent long-term disease complications.

Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group; Jacobson AM, Musen G, Ryan CM, Silvers N, Cleary P, Waberski B, Burwood A, Weinger K, Bayless M, Dahms W, and Harth J: Long-term effect of diabetes and its treatment on cognitive function. N Engl J Med 356: 1842-1852, 2007.

Omega-3 Fatty Acids Reduce Children's Risk of Developing Early Markers of Type 1 Diabetes: Type 1 diabetes is a complex disease to which both genetic factors and environmental triggers contribute. Although several genes associated with type 1 diabetes have been identified, much less is known about environmental factors that increase or decrease a person's risk of developing the disease. Previous studies in mice and humans suggested that omega-3 fatty acids may be protective. These fatty acids are found in some food sources, such as fish, green leaves of plants, and some seeds, nuts, and legumes. To examine the effect of dietary omega-3 fatty acids on the risk of developing type 1 diabetes, researchers studied participants in the Diabetes Autoimmunity Study of the Young (DAISY). This longitudinal, observational study enrolled and followed children at high genetic risk of developing type 1 diabetes. At different time intervals, the researchers measured the children's levels of predictive markers of future development of type 1 diabetes (autoantibodies). The scientists examined whether there was a correlation between omega-3 fatty acid intake and the development of early risk markers of type 1 diabetes (defined by the presence of at least two different predictive autoantibodies). They collected data on omega-3 fatty acid intake from an annual questionnaire in which parents were asked about their children's food intake beginning at age 2 and by measuring a biological marker of fatty acid levels. The researchers discovered that children with higher reported dietary intake of omega-3 fatty acids were less likely to develop early markers of type 1 diabetes. However, correlations found in observational studies are not sufficient to establish a causal relationship. Such proof requires a clinical trial. Recently, Type 1 Diabetes TrialNet launched a pilot study to test whether omega-3 fatty acid supplements given to pregnant women in their third trimester, or added to babies' formula, could protect children from developing early markers of type 1 diabetes. If successful, dietary intervention to increase children's levels of omega-3 fatty acids could be a possible approach for preventing type 1 diabetes.

Norris JM, Yin X, Lamb MM, Barriga K, Seifert J, Hoffman M, Orton HD, Barón AE, Clare-Salzler M, Chase HP, Szabo NJ,

Erlich H, Eisenbarth GS, and Rewers M: Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. JAMA 298: 1420-1428, 2007.

Increasing Rates of Childhood Diabetes: Worldwide data suggest that rates of type 1 diabetes are increasing. Now, similar observations are being made in the United States. Recently, researchers participating in a study, called SEARCH for Diabetes in Youth, reported the prevalence (or total number of cases) of childhood diabetes in the U.S. One of every 523 youth had physician-diagnosed diabetes in 2001. To further characterize diabetes in children, SEARCH investigators next examined the incidence (or number of new cases) of type 1 and 2 diabetes in youth under age 20 in 10 locations across the U.S. for the 2002-2003 period. Non-Hispanic white youth had the highest rate of childhood diabetes of all racial and ethnic groups. The incidence rate was highest among 10- to 14-year-old youth and slightly higher in females than in males. While type 2 diabetes is increasing in children over 10, particularly in minority populations, type 1 diabetes accounts for most new cases, with an estimated 15,000 youths diagnosed annually. In a separate study, researchers determined that not only is the incidence of type 1 diabetes increasing, but it is occurring at younger ages and has particularly increased in children under age four. A study in Colorado found that rates of the disease doubled in these young children from the 1980s compared to 2002-2004. Overall, rates of type 1 diabetes in Colorado's youth increased by 1.6-fold over this time period. Together, these studies suggest that type 1 diabetes is increasing in the U.S., particularly in the youngest children. Further understanding of these trends can help to uncover the underlying mechanisms that are driving them.

Writing Group for the SEARCH for Diabetes in Youth Study Group, Dabelea D, Bell RA, D'Agostino RB Jr, Imperatore G, Johansen JM, Linder B, Liu LL, Loots B, Marcovina S, Mayer-Davis EJ, Pettitt DJ, and Waitzfelder B: Incidence of diabetes in youth in the United States. JAMA 297: 2716-2724, 2007.

Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith G, Bloch C, Rewers M, and Dabelea D: Increasing incidence of type 1 diabetes in 0- to 17-year-old Colorado youth. Diabetes Care 30: 503-509, 2007.

PANCREATIC REGENERATION

Finding safe and effective ways to increase the mass of insulin-producing beta cells could greatly benefit diabetes patients. These cells, which are found in clusters called "islets" in the pancreas, are damaged in both type 1 and type 2 diabetes. It is important to understand the underlying mechanisms controlling beta cell growth and development in order to identify possible therapeutic targets and preserve or restore beta cell function.

Expanding Beta Cell Mass: Researchers have determined that in mice, a transcription factor protein called HNF-4alpha is important for expanding beta cell mass during pregnancy. Pregnancy is a period when the body becomes somewhat less sensitive to insulin and therefore needs more insulin production. The researchers generated a genetically-engineered mouse model, in which most of the animals' beta cells no longer produced this protein. Experiments using these mice demonstrated that the protein regulates the expression of several genes, many of which are involved in cell growth. Surprisingly, young adult genetically-engineered mice did not appear to have decreased beta cell mass, which would be expected if the protein regulated beta cell growth. However, the researchers found that during pregnancy the animals' beta cell mass was lower than that of controls. This research suggests that HNF-4alpha plays an important role in regulating beta cell expansion during this key period. Mutations in the gene encoding this protein were previously found to be associated with a rare and inherited form of diabetes, called MODY1. However, this research is the first to suggest that HNF-4alpha is necessary for expanding beta cell mass. Because it was particularly important during pregnancy in the animal model, it is possible that stimulation of the protein could one day be a viable approach to treat gestational diabetes. Additional research will provide greater understanding of this protein's role in diabetes, including its role in increasing beta cell mass in response to other stressors such as age and other forms of insulin resistance, and may lead to novel therapeutic approaches for increasing beta cell mass in people with the disease.

Gupta RK, Gao N, Gorski RK, White P, Hardy OT, Rafiq K, Brestelli JE, Chen G, Stoeckert CJ Jr, and Kaestner KH: Expansion of adult beta-cell mass in response to increased metabolic demand is dependent on HNF-4alpha. Genes Dev 21: 756-769, 2007.

New Mouse Model for Studying Beta Cell Regeneration: Finding a way to regenerate the beta cells of the pancreas is a possible approach for treating both type 1 and type 2 diabetes. Scientists in the NIDDK-supported Beta Cell Biology Consortium have now developed a mouse model useful for studying beta cell regeneration. When the genetically engineered mice are treated with a certain drug, a toxin is expressed in their beta cells. Expression of the toxin causes the beta cells to die, and the mice develop diabetes. Surprisingly, the researchers found that if they stopped the drug treatment after the mice developed diabetes, the animals recovered from the disease. The mice not only regained normal blood sugar levels, but also regenerated their beta cell mass. The scientists determined that the new beta cells came predominantly from preexisting beta cells, suggesting that beta cells have a significant capacity for regeneration.

Importantly, the mouse model also provides a system for testing the effects of different drugs on beta cell regeneration. For example, transplanting pancreatic islets that contain beta cells is an approach being tested for treating type 1 diabetes in people. However, the transplanted islets often lose function in the patients over time. Using the mouse model, the researchers showed that drugs commonly used to suppress the immune system after islet transplantation have an adverse affect on beta cell regeneration. This result may help to explain why the transplanted islets lose function in people. The mouse model may be useful for identifying other immunosuppressive drugs that do not have this negative effect on beta cell regeneration. This research suggests that finding ways to promote regeneration of existing beta cells may be a therapeutic approach for treating diabetes. It also provides an important model system for testing the effect of therapeutic agents on beta cell regeneration.

Nir T, Melton DA, and Dor Y: Recovery from diabetes in mice by beta cell regeneration. J Clin Invest 117: 2553-2561, 2007.

REGULATORS OF METABOLISM IN HEALTH AND DISEASE

Controlling Inflammation and Metabolism—The STAMP2 Protein: Chronic overeating can lead to obesity and type 2 diabetes, both of which are associated with excess inflammation and its damaging effects. However, normal daily fluctuations in nutrient levels do not incite inflammatory responses, and scientists recently gained insight as to why. A protein called STAMP2, made primarily by fat cells, keeps such inflammation in check. The scientists began with the hypothesis that there must be some way that the body limits inflammation, for example, when there is temporary nutrient abundance from eating. They thus designed a screen in mice for biologic factors that might play a role in this process, and uncovered STAMP2 as a likely candidate. When the researchers compared fed and fasted mice, they found that STAMP2 levels varied in response to changes in nutrient levels. In obese mice, however, this regulation of STAMP2 by nutrients was lost. The researchers then generated mice that lacked STAMP2, and observed substantial inflammation in visceral fat, the type of fat that is located around the internal abdominal organs and is most strongly associated with metabolic disease. To examine more specifically the role of STAMP2 in nutrient-rich conditions, the scientists injected mice with fat (in a form used for intravenous nutrition) and glucose, and found that these nutrients elicited more inflammation in STAMP2-deficient mice than in normal mice. Mice without STAMP2 also showed signs of insulin resistance, a condition that both precedes and characterizes type 2 diabetes, and the insulin resistance was observed even though the mice were fed a standard diet. Yet another adverse health effect observed in mice without STAMP2 was fatty liver disease. Collectively, these results illuminate a new regulatory link between nutrient metabolism and inflammation. Levels of STAMP2 in mice increase in response to nutrients and inflammatory signals, and this protective protein in turn limits inflammation under normal conditions. In obesity and diabetes, however, STAMP2 action may be disrupted or insufficient to prevent chronic inflammation. Future research will help elucidate the role of STAMP2 in human metabolism and disease.

Wellen KE, Fucho R, Gregor MF, Furuhashi M, Morgan C, Lindstad T, Vaillancourt E, Gorgun CZ, Saatcioglu F, and Hotamisligil GS: Coordinated regulation of nutrient and inflammatory responses by STAMP2 is essential for metabolic homeostasis. Cell 129: 537-548, 2007.

Discovery of a Biological Missing Link May Aid Research on Diabetes and Obesity: Researchers have identified a hormone that helps animals adapt to periods of starvation and could play a future role in treating obesity and diabetes. When food is plentiful, the body uses the energy it gets from food as its main fuel source. However, during periods of starvation, the body uses other metabolic pathways to generate energy. For example, the liver metabolizes fatty acids from fats stored in adipose tissue to produce "ketone bodies," which the body (particularly the brain) uses for energy. Mammals may also enter into hibernation-like states to conserve energy. It is important for individuals that these processes are tightly regulated in order to stay healthy. Therefore, scientists are examining the metabolic cues that regulate how the body transitions from the "fed" to the "fasting" state.

Previous research determined that a transcription factor, called PPAR-alpha, plays an important role in the body's metabolic response to starvation. Research now shows that this transcription factor increases the levels of a hormone, called FGF21, which is produced and secreted by the liver. In a mouse model, levels of the hormone increased after a fast. In addition, researchers discovered that the hormone regulates key metabolic pathways needed to adapt to starvation in mice engineered to express high levels of the hormone. For instance, it promotes the breakdown of fatty acids and increases the production of ketone bodies. It also caused the animals to go into a hibernation-like state to further conserve energy. In a complementary study, researchers studied mice that were experimentally induced to have low levels of FGF21 in their livers. When placed on a special diet that simulates nutrient availability in the fasted state and promotes lipid metabolism in the liver, the animals had severe metabolic abnormalities that included fatty liver and high levels of fats circulating in the blood. Thus, the hormone is required for the liver to fully regulate lipid metabolism under these nutritional demands. In previous research, the hormone was found to improve metabolic characteristics of diabetic and obese mice. Together, these research studies suggest that FGF21 is a missing link between diet and the regulation of lipid metabolism in the liver during fasting. They also suggest that the hormone could be a possible therapeutic target for treating diabetes and obesity.

Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, and Kliewer SA: Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 5: 415-425, 2007.

Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, and Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 5: 426-437, 2007.

The Skeleton Plays More Than a Supporting Role: Researchers have shown that the skeleton—once thought to be strictly structural in purpose—is an endocrine organ that may play an important role in obesity and diabetes. In earlier studies, leptin, a hormone produced by fat cells and involved in regulating body weight, was found to send chemical signals to bone. In biology, communication is often a two-way street, so researchers hypothesized that bone might be sending its own signals back to the body. To examine this possibility, scientists generated a genetically-engineered mouse model in which bone cells lacked a protein, called OST-PTP. Unexpectedly, mice lacking this protein were protected from both diabetes and obesity. The animals also had several metabolically favorable characteristics: increased numbers of insulin-producing beta cells in the pancreas, lower blood sugar levels, increased insulin secretion, and increased insulin sensitivity. The animals expended more energy and had less visceral fat compared to their control counterparts.

How is bone causing these favorable metabolic changes? The scientists discovered that OST-PTP regulates the activity of a hormone, called osteocalcin, that is produced and secreted by bone-forming cells. Osteocalcin was found to regulate both insulin secretion and insulin sensitivity. It also increases the mass of insulin-producing beta cells. Without this hormone, animals are obese, are insulin resistant, and have decreased beta cell mass. This research is the first to demonstrate that bone has an important role in regulating metabolism and weight. It also sheds new light on the link between diabetes and obesity. Researchers have already observed that people with type 2 diabetes have low osteocalcin levels. If osteocalcin has a similar role in humans, the identification

of therapeutic agents to intervene in this signaling pathway could open new doors to therapy. Now that we know that bone is communicating with endocrine organs in the body, a closer listen could uncover other exciting findings.

Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, and Karsenty G: Endocrine regulation of energy metabolism by the skeleton. Cell 130: 456-469, 2007.

Shedding LIGHT on Regulation of Lipid Metabolism: Recent studies have shown that two chemicals in the immune system that can promote inflammation also play an important role in controlling lipid (fat) metabolism. Elevated blood levels of lipids, such as triglycerides, and cholesterol are associated with atherosclerosis (clogging of the blood vessels) and can contribute to heart disease and stroke. Previous research has elucidated how the immune system and elevated blood lipids contribute to the inflammation and plaque formation characteristic of atherosclerosis. Now, new studies have added a surprising twist to this tale by showing how the immune system also acts to directly regulate lipid metabolism. Building on previous findings from human and animal studies, researchers studied the effects on lipid metabolism of two immune-system chemicals that promote inflammation—called lymphotoxin and LIGHT. These chemicals are produced on the surface of immune cells known as T cells. Using mice genetically altered to overproduce LIGHT, researchers found that an overabundance of this chemical on the mouse T cells correlated with elevated cholesterol and triglycerides in the blood. Because the liver is an important player in lipid metabolism, the researchers next conducted a microarray analysis of 45,000 genes expressed in the liver of the LIGHT-overproducing mice. They noticed very low levels of the enzyme hepatic lipase, which usually contributes to lipid metabolism by breaking down triglycerides. Levels of this enzyme were 20 times lower in the mice with excessive LIGHT than in normal mice. Injecting the mice with a compound to neutralize the overactive LIGHT production corrected the lipid imbalances. Further studies in other animal models and in cell culture provided more evidence that, in addition to their pro-inflammatory role in the immune system, LIGHT and lymphotoxin work through the same pathway to inhibit a beneficial liver enzyme and contribute to a pathologic elevation of lipid levels. This work highlights a previously unappreciated relationship between immune cells and liver cells in controlling lipid metabolism. Furthermore, the research demonstrates the feasibility of a possible therapeutic approach for treating atherosclerosis by targeting the LIGHT-lymphotoxin pathway in the immune system.

Lo JC, Wang Y, Tumanov AV, Bamji M, Yao Z, Reardon CA, Getz GS, and Fu YX: Lymphotoxin beta receptor-dependent control of lipid homeostasis. Science 316: 285-288, 2007.

Metabolic Connection Found Between Brain Glucose and Liver Fat Processing: Recent research findings point to a previously unrecognized relationship between metabolism of glucose in the brain and the processing and distribution of lipids by the liver. Some of the defining features of metabolic syndrome—a constellation of symptoms that puts individuals at higher risk for developing diseases such as type 2 diabetes and cardiovascular disease—are a higher level of fats known as triglycerides in the blood, and overweight. The liver packages triglycerides (TGs) into particles known as very low-density lipoprotein (VLDL) for secretion into the blood. This process is regulated by such factors as nutrients and the hormone insulin. In the current study, researchers provided a new insight into this process by showing how the brain's metabolic responses to nutrient intake can communicate with the liver, affecting its processing and packaging of fats. To achieve this, the researchers first directly infused glucose into the brains of rats and observed a resulting decrease in the level of circulating TGs. Then, by using specific enzymatic inhibitors and metabolic products, they identified the major components in the pathway that leads from brain glucose metabolism to effects on liver VLDL-TG (fat) secretion. Ultimately, the researchers demonstrated the relevance of this brain-liver metabolic connection to situations such as metabolic syndrome through experiments on rats overfed a high-fat diet. When glucose was infused into the brains of these rats, the typical reduction in circulating VLDL-TG was not seen, suggesting that overfeeding disrupts the brain-liver connection, in terms of its ability to harmoniously regulate metabolism. These findings of

a new brain-liver circuit that regulates fat distribution, which could be defective in those with metabolic syndrome, provide a possible target for future approaches to controlling this complex condition.

Lam TKT, Gutierrez-Juarez R, Pocai A, Bhanot S, Tso P, Schwartz G, and Rosetti L: Brain glucose metabolism controls the hepatic secretion of triglyceride-rich lipoproteins. Nat Med 13: 171-180, 2007.

Metabolic Regulator May Also Be a Therapeutic Target for Degenerative Brain Diseases: To drive its essential activities, the body relies on mitochondria, the so-called "power-houses" of the cell. Through a series of chemical reactions, mitochondria convert nutrients from food into usable energy and heat. However, these reactions sometimes generate dangerous chemical by-products that can damage cells in the brain, heart, and other vital organs. This type of damage, called oxidative damage, is thought to play a role in degenerative brain diseases, such as Huntington's disease, Parkinson's disease, and Alzheimer's disease. Scientists now have evidence that a key regulator of mitochondrial activity also regulates cellular defenses against oxidative damage. Through experiments using cell lines and rodent models, the researchers found that the PGC-1alpha protein is necessary for cells and tissues to react normally to the threat of oxidative damage. Cells with increased levels of PGC-1alpha were more strongly protected against oxidative damage. Conversely, cells lacking the protein were unable to increase production of enzymes that "clean up" the toxic molecules and render them harmless. Most intriguingly, the researchers found that mice genetically engineered to lack PGC-1alpha had brains more vulnerable to oxidative damage than those of normal mice. PGC-1alpha has been well-studied because reduced levels are thought to play an important role in diabetes and obesity, and molecules that increase its activity are in development. While the connection between brain degeneration and PGC-1alpha activity needs further exploration, current research indicates that this protein may be a promising therapeutic target not only for metabolic disorders, but for brain diseases as well.

St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, and Spiegelman BM: Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 127: 397-408, 2006.

Type 1 Diabetes TrialNet: Study Testing Oral Insulin To Prevent Type 1 Diabetes

Researchers have begun a clinical study to test the ability of oral insulin to prevent or delay type 1 diabetes in at-risk people. NIDDK's Type 1 Diabetes TrialNet is a network of researchers dedicated to the understanding, prevention, and early treatment of type 1 diabetes. TrialNet is conducting the study in more than 100 medical centers across the U.S., Canada, Europe, and Australia.

In type 1 diabetes, a person's own immune system destroys the insulin-producing beta cells of the pancreas. Beta cells sense blood glucose and produce the hormone insulin, which is essential to regulating glucose and converting it to energy. The immune attack on beta cells begins well before a person develops type 1 diabetes and continues long after the disease is diagnosed. In the early stages of disease, up to 10 years before diabetes is diagnosed, proteins called "autoantibodies" may appear in the blood. These autoantibodies to glutamate decarboxylase (GAD), IA-2, and to insulin itself indicate a greater risk for developing type 1 diabetes.

In the new study, researchers are testing whether an insulin capsule taken by mouth once a day can prevent or delay diabetes in a specific group of people at risk for type 1 diabetes. An earlier trial suggested that oral insulin might delay type 1 diabetes for about 4 years in some people with high levels of insulin autoantibodies. Animal studies have also suggested that insulin taken orally may prevent type 1 diabetes. Some scientists think that introducing insulin via the digestive tract induces tolerance, or a quieting of the immune system. Insulin taken orally has no side effects because the digestive system breaks it down quickly. To lower blood glucose, insulin must be injected or administered by an insulin pump.

Management of type 1 diabetes is extremely burdensome to patients and their families. People with the disease must check their blood sugar levels multiple times per day with a finger stick, administer insulin, and monitor food intake and physical activity levels. Moreover, patients are susceptible to developing long-term disease complications affecting the eyes, kidneys, nerves, and heart. Therefore, identifying ways to prevent the disease is critically important, not only to prevent these serious complications, but to spare people from the tremendous burden of daily disease management.

In addition to conducting the new oral insulin prevention trial, TrialNet also studies therapies to preserve insulin production in people recently diagnosed with type 1 diabetes. For example, one TrialNet study seeks to turn off the immune attack on beta cells with rituximab, a monoclonal antibody that binds to and temporarily destroys a specific class of immune cells.

TrialNet is currently recruiting people to participate in its studies. For more information about enrolling in TrialNet studies, please visit *www.DiabetesTrialNet.org* or call *1-800-HALT-DM1 (1-800-425-8361)*.

People with Diabetes and Sickle Cell Trait Should Have Reliable A1C Test: Campaign Informs Physicians and Patients

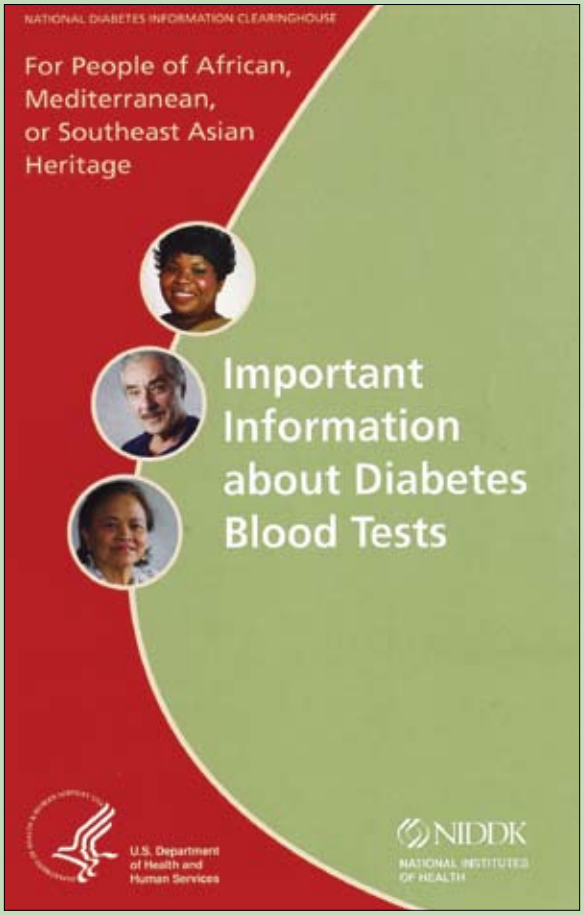
A new NIDDK information campaign highlights the importance of using accurate methods to test hemoglobin A1c in people with diabetes who have sickle cell trait or other inherited forms of variant hemoglobin. The specific needs for testing blood glucose control in these patients are explained in two booklets, "Sickle Cell Trait and Other Hemoglobinopathies and Diabetes: Important Information for Physicians" and "For People of African, Mediterranean, or Southeast Asian Heritage: Important Information about Diabetes Blood Tests" from NIDDK's National Diabetes Information Clearinghouse at *www.diabetes.niddk.nih.gov*

Studies have repeatedly shown that intensive control of blood glucose, blood pressure, and cholesterol reduces heart disease and the other complications of diabetes. The hemoglobin A1c blood test (or simply the A1C test) is an essential tool in diabetes care because it shows a patient's average level of blood glucose control in the previous 2 to 3 months. Physicians base their treatment decisions in large part on the A1C test results. Inaccurate A1C readings, whether falsely high or low, may lead to the over treatment or under treatment of diabetes.

The A1C test, though essential in diabetes management, is not recommended for diagnosing diabetes. However, if an A1C test is given to a person not known to have diabetes and the result is higher than normal, a fasting blood glucose test is needed to confirm a diabetes diagnosis.

The National Glycohemoglobin Standardization Program (NGSP) at the University of Missouri School of Medicine, supported by the NIDDK and Centers for Disease Control and Prevention, is working to improve and standardize the measurement of A1C in laboratories around the world. The NGSP website (*www.NGSP.org*) lists the test methods that accurately measure A1C in patients with hemoglobin variant S, also known as sickle cell trait, and variant C, another common variant in the United States.

In a press release announcing the new campaign, Dr. Randie Little, head of the NGSP, said, "In the United



This booklet, which was developed for people of African, Mediterranean, or southeast Asian descent, is part of a new NIDDK campaign that highlights the importance of using accurate methods to test hemoglobin A1c in people with diabetes who have sickle cell trait or other inherited forms of variant hemoglobin.

States, more than 3,000 labs rely on 20 different methods to measure A1C in people with diabetes. However, six of these methods yield unreliable results in patients with sickle cell trait. Health care professionals caring for people with diabetes should know that specific A1C tests should be used in this group of patients."

Many individuals are unaware they have a hemoglobin variant such as sickle cell trait because the condition usually

causes no symptoms. In diabetes patients of African, Mediterranean, or southeast Asian descent, several situations may suggest the presence of a hemoglobin variant:

- An A1C result does not correlate with results of self blood glucose monitoring;
- An A1C result is different than expected or radically differs from a previous test result after a change in lab A1C methods; or
- An A1C result is more than 15 percent.

In the same announcement, Dr. Griffin Rodgers, NIDDK Director, advised physicians, "If you see a significant discrepancy between a patient's A1C reading and the results of routine blood glucose monitoring, consider the possibility that your patient may have a hemoglobin variant and find out if your lab is using an accurate method to measure A1C."

Hemoglobin is the oxygen-transporting protein in red blood cells. Mutations in the genes that code for the protein, which occur more frequently in people of African, Mediterranean, and southeast Asian descent, cause variations in the structure or amount of hemoglobin. Researchers have identified hundreds of hemoglobin variants in the human population, affecting millions of people worldwide.

The most common variant is sickle cell trait in which a person inherits a gene for hemoglobin S and a gene for hemoglobin A, the usual form of hemoglobin. Sickle cell trait affects about 8 percent of African Americans.[1] Having sickle cell trait or another hemoglobin variant does not increase a person's risk for developing diabetes.

In sickle cell disease, a person inherits two genes for hemoglobin S, which causes the malformation, or sickling, of red blood cells, leading to anemia, repeated infections, and periodic episodes of pain. The A1C test is not used in diabetes patients with sickle cell anemia due to the shortened life span of red blood cells.

Diabetes afflicts nearly 21 million people in the United States, but its burden is disproportionately felt by minorities, including African Americans, Hispanic/Latino Americans, American Indians and Alaska Natives, Asian Americans, and Pacific Islanders. About 13 percent of African Americans age 20 and older suffer from diabetes, a rate that is nearly twice that of non-Hispanic whites.[2]

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[1] *www.nhlbi.nih.gov/health/dci/Diseases/Sca/SCA_WhoIsAtRisk.html*
[2] *www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf*

STORY OF DISCOVERY

Studies of Underlying Biology of Insulin Secretion Pave the Way to New Treatment for Neonatal Diabetes

In the 1950s, little did scientists know that new drugs being used to treat type 2 diabetes in adults would be used half a century later to treat a rare form of diabetes in babies.

The drugs are called "sulfonylureas." The initial obser vation about these drugs came in the early 1940s, when French scientists used them to treat typhoid patients. After treatment, the patients had symptoms of dangerously low blood sugar levels (hypoglycemia). Tests of the same drugs in dogs showed that they stimulate insulin secretion, leading to hypoglycemia. Insulin is a hormone released by the beta cells of the pancreas in response to elevated blood sugar levels. Its release then promotes the uptake of sugar by cells in the body. These novel findings paved the way to clinical trials to test this class of drugs in people with the form of diabetes now known as type 2. People with type 2 diabetes produce insufficient amounts of insulin to compensate for diminished responsiveness of cells to the hormone. In the mid-1950s, sulfonyl-ureas were found to be effective for treating human type 2 diabetes. Sulfonylureas were the first diabetes pills used as an alternative to insulin injections for people with type 2 diabetes.

This result was great news, but it remained unknown exactly *how* sulfonylureas stimulated insulin secretion. Teasing out this mystery has been the subject of research for several decades. A clue came in 1985, when NIDDK-supported scientists demonstrated that sulfonylureas inhibit a potassium ion channel. This type of channel allows movement of potassium ions between the inside and outside of the beta cell, a common method the cell uses to control its processes. Although this observation shed some light on how the drugs may work, it also led to several new questions: what protein (or proteins) comprised the potassium ion channel? Were sulfonylureas binding directly to the channel or were they binding to an intermediate protein to stimulate insulin release?

A breakthrough came in 1995, when NIDDK-supported scientists cloned a gene encoding the sulfonylurea receptor, or SUR. This protein was the target to which the drugs were binding in beta cells. Interestingly, mutations in the gene encoding SUR were found to be linked to a rare genetic disease, called persistent hyperinsulinemic hypoglycemia of infancy (PHHI). People with this disease have high levels of insulin and correspondingly low blood sugar levels. These findings suggested that SUR was a critical component of cellular pathways regulating insulin secretion. Was SUR the potassium ion channel that was inhibited by sulfonylureas? Research showed that SUR alone could not function as an ion channel. However, a few months later, the same group of NIDDK-supported scientists identified SUR's partner—a protein called Kir6.2. The combination of SUR and Kir6.2 worked together as a potassium ion channel. This research demonstrated that the previously unidentified potassium ion channel was composed of SUR and Kir6.2; sulfonylureas bound directly to the SUR subunit of the channel to inhibit it.

These pioneering NIDDK-supported discoveries contributed to a model of the regulation of insulin secretion by sugar. The SUR/Kir6.2 ion channel regulates the balance of potassium and calcium ions inside and outside the beta cell, which in turn helps to regulate insulin secretion. In healthy people, when blood sugar levels are low, the channel is "open" and insulin is not secreted. When blood sugar levels are high (e.g., after a meal), sugar metabolism in the beta cell closes the ion channel, and insulin is secreted. Sulfonylureas cause the same biological effect as high

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sugar levels—they close the channel and stimulate insulin release from the beta cell. Mutations causing PHHI also result in a similar biological effect—they prevent the channel from opening and promote insulin release.

Building on this research foundation, researchers in Europe hypothesized that SUR/Kir6.2 may be involved in monogenic diabetes. These forms of diabetes result from mutations in a single gene; in contrast, type 1 and type 2 diabetes involve variations in multiple genes. The two main forms of monogenic diabetes are neonatal diabetes mellitus and maturity-onset diabetes of the young (MODY). Neonatal diabetes is a rare condition, usually occurring within the first 6 months of life, and may either be permanent, or transient—with the possibility of relapse later in life. While a number of genes had been found that each could cause MODY—the monogenic form of diabetes that is usually diagnosed later in childhood or young adults—the genetic cause of permanent neonatal diabetes was unknown.

In 2004, the European researchers examined the gene encoding Kir6.2 in patients with permanent neonatal diabetes. Several people with the disease had mutations in this gene. Upon examination of the underlying biology of the mutant channels, researchers found that the mutations caused the channels to be "open" all the time, even in the presence of high levels of sugar. Thus, these mutations appeared to prevent insulin release. These findings helped to explain why children with these mutations produce insufficient amounts of insulin and require insulin administration. They also suggested that, if there were another way to close down the channels—such as through treatment with sulfonylureas—perhaps insulin secretion could be restored.

These observations laid the foundation for a recent clinical trial by the same group of scientists to test the effect of switching neonatal diabetes patients from insulin to oral sulfonylurea treatment. People in the trial had mutations in their gene encoding Kir6.2. Strikingly, 90 percent of patients successfully discontinued insulin after receiving the oral drugs. Average blood sugar control improved in all patients who switched treatment strategies. These results are extremely exciting because oral therapy is a much less burdensome treatment strategy than insulin administration, which requires daily injections or use of a pump. It is of particular benefit for babies and young children to be able to take oral medication for their neonatal diabetes, rather than experience an arduous regimen of daily insulin administration.

NIDDK-supported scientists have also recently shown that some people with permanent and transient neonatal diabetes have mutations in their gene encoding SUR. People with these mutations who were switched from insulin to sulfonylurea therapy appeared to respond favorably. Together, these studies demonstrate that mutations in the gene encoding Kir6.2 are the most common cause of permanent neonatal diabetes; mutations in the gene encoding SUR account for fewer cases of permanent and some cases of transient neonatal diabetes.

How do people with PHHI and neonatal diabetes have mutations in the same ion channel, but PHHI patients have too much insulin and patients with neonatal diabetes have insufficient amounts? It turns out that mutations causing PHHI have the opposite effect on the function of the ion channel than mutations causing neonatal diabetes. The mutations causing PHHI prevent the channel from opening, which causes beta cells to secrete insulin continually. The mutations causing neonatal diabetes cause the channels to always remain open, which prevents insulin release. Thus, even though the same ion channel is involved in both diseases, the effects of the different mutations lead to completely opposite biological responses.

These studies identify a novel mechanism for the development of a significant fraction of permanent and transient neonatal diabetes mellitus and identify

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a less burdensome treatment strategy for some patients. They also pave the way for genetic testing to inform personalized treatments for people with the disease. Importantly, this research elegantly demonstrates how long-term studies of underlying biological mechanisms directly led to an improved treatment option for patients. Incremental studies of how sulfonylureas worked in the beta cell culminated with the NIDDK-supported discovery of the SUR/Kir6.2 potassium ion channel. This discovery not only informed key genetic studies, but also provided a much greater understanding of the basic biology of insulin secretion. Recently, using a whole genome association study, NIH-supported investigators have confirmed that the gene for Kir6.2 can contribute to type 2 diabetes. These studies now serve as the foundation for additional research on the role of this ion channel in diabetes, with the potential to improve and personalize therapies by targeting specific treatments to patients with specific genetic changes underlying their diabetes.

Genetic testing could be helpful in selecting the most appropriate treatment for people with monogenic diabetes. If you think that you or a family member has a monogenic form of diabetes, you should seek help from a healthcare provider. For more information on monogenic forms of diabetes, please see an NIDDK fact sheet available at: *http://diabetes.niddk.nih.gov/dm/pubs/mody/mody.pdf*

The Intersection of Drug Metabolism and Diabetes

Dr. David Moore

Dr. David D. Moore is a professor in the departments of Molecular and Cellular Biology and Molecular and Human Genetics at Baylor College of Medicine in Houston, Texas. He received his Ph.D. in Molecular Biology from the University of Wisconsin, Madison. He trained as a post-doctoral fellow in the laboratory of Dr. Howard Goodman at the University of California, San Francisco. He has received numerous awards for his teaching and research, including the Edmund B. Astwood Award from the Endocrine Society. The following are highlights from the scientific presentation that Dr. Moore gave to the NIDDK's National Advisory Council in May 2007.

Dr. Moore's presentation focused on nuclear hormone receptors, which are proteins that help certain chemicals, such as vitamin D or the sex hormones estrogen and testosterone, exert some of their effects. Typically, when one of these chemicals enters a cell it binds to its receptor, and the hormone-receptor complex then interacts with both specific DNA sites and other proteins to turn some genes in the nucleus "on" and others "off." Most of the 48 nuclear hormone receptors encoded by the human genome actually bind—not to true hormones—but to other chemicals coming from outside the body. Through support from NIDDK, the National Heart, Lung, and Blood Institute and the National Institute of Environmental Health Sciences, the Nuclear Receptor Signaling Atlas project is cataloging the diverse and extremely important physiological effects of these proteins. An important subset of these receptors plays a vital role in triggering the process by which the body eliminates certain drugs or toxins, a process called drug metabolism.

Dr. Moore first discussed recent research from his laboratory that has shed light on the way that type 1 diabetes influences drug metabolism via CAR, a member of the nuclear hormone receptor family origi nally cloned in his laboratory. Dr. Moore then presented work showing that drug metabolism mediated by CAR may be helpful in achieving healthier blood glucose levels in patients with type 2 diabetes. Thus, not only can diabetes influence drug metabolism, but also drug metabolism can affect diabetes.

Diabetes Impacts Drug Metabolism

The experiments Dr. Moore highlighted were inspired by observations from other researchers that type 1 diabetes accelerates metabolism of certain drugs, both in humans and in rodent models. Dr. Moore's lab looked at drug metabolism genes that are turned on by CAR. The researchers found that these genes were turned on in a mouse model of type 1 diabetes. Controlling the diabetes reversed the effect: when insulin was given to the mice, the CAR-induced genes turned off.

In fact, type 1 diabetes not only leads to activation of drug metabolic genes, but also has a profound effect on the metabolism of certain drugs. Mice with induced type 1 diabetes rapidly clear their systems of a compound that induces temporary paralysis, while normal mice cannot. These experiments also underline the central importance of the CAR nuclear receptor in affecting drug metabolism: mice without CAR take much longer to clear the drug, whether or not they have diabetes.

How does CAR promote speedier drug metabolism in animals or people with diabetes? The answer may have to do with an unusual property of CAR compared to other nuclear hormone receptors. In addition to its response to binding to a hormone or other activating molecule from outside the cell, CAR can move into the

nucleus and turn on its target genes when it is activated by an enzyme within the cell called AMP kinase. Not surprisingly, AMP kinase is activated by certain drugs. In addition, the Moore lab found it to be modestly activated in the livers of mice with uncontrolled type 1 diabetes. Further research will be required to extend these suggestive results and determine the actual mechanism underlying the impact of type 1 diabetes on drug metabolism.

Drug Metabolism Impacts Diabetes

Early in the course of type 2 diabetes, the pancreas reacts to elevated blood glucose by producing more insulin to try to compensate. However, because the disease is characterized by insulin resistance, the result is that both blood sugar and insulin are elevated in these patients if they do not have proper treatment. Gradually, during the course of the disease, years of elevated blood glucose take their toll on insulin-producing cells, diminishing their ability to produce the vital hormone. Thus, without proper treatment, glucose control often goes from bad to worse.

Surprisingly, researchers have found that phenobarbital, a medication formerly used to treat epilepsy, also has the effect of reducing blood glucose levels in people with type 2 diabetes. The effect is only observed in patients whose disease is in its early stages and whose ability to produce insulin is not yet seriously diminished or lost. Phenobarbital has no impact on blood glucose or insulin levels either in those whose type 2 diabetes has progressed to the point where insulin production falls, or in people who do not have the disease.

Phenobarbital is an effective treatment for epilepsy, but it is no longer widely used because it has two serious side effects: it is a potent sedative, and it is such a powerful activator of drug metabolism (via CAR) that it can adversely affect the way a patient's other medications are handled by the body. The effect on blood glucose can also be thought of as a side effect, albeit a potentially beneficial one in some patients due to its theoretical benefit for some people with type 2 diabetes. However, because of its serious side effects and the availability of safer medications that improve insulin sensitivity, phenobarbital is not a recommended treatment for the disease.

Nevertheless, understanding the way phenobarbital exerts its effects might point the way to other avenues of diabetes treatment. The lower blood glucose levels observed in type 2 diabetes patients taking phenobarbital might result from an overall improvement in insulin sensitivity among the body's cells that allows them to absorb more glucose, or they might come from a more subtle metabolic effect.

To distinguish between these possibilities, the Moore lab examined a strain of mice that are obese because they lack a key hormonal mediator of appetite control. Invariably, the uncontrolled appetite of these mice leads them to become obese and to develop type 2 diabetes at a very young age. As in humans, a drug treatment that stimulates CAR helped normalize the otherwise sharply elevated blood glucose levels typically observed in these animals. When the obese mouse strain was modified by deleting the gene for CAR, the drug treatment had no effect on blood glucose, indicating that CAR is necessary for the effect.

If CAR has a general effect on insulin sensitivity, CAR stimulation would be expected to improve the ability of all tissues in the animal to absorb glucose. However, when the Moore group looked more carefully at how specific tissues in these mice respond to a sudden surge of injected glucose, they discovered this was not the case. CAR stimulation did not improve glucose clearance in peripheral tissues. Rather, the effect was confined to the liver.

One possible explanation for the importance of the liver in mediating CAR's glucose-modulating effects relates to the liver's vital metabolic role in keeping blood glucose sufficiently high to facilitate brain function during periods of fasting. The liver does this

by liberating glucose from energy stores. One of the important messages insulin sends to the body is to tell the liver that it should stop producing glucose when blood levels of the molecule begin to rise after a meal, and to signal to the body that it should instead switch to replenishing energy stores. Because of the insulin resistance observed in type 2 diabetes, however, the liver continues to produce glucose even when its concentration in the blood is already too high. Interestingly, CAR seems to reduce the activities of several proteins with a key function in liver glucose production, while stimulating proteins that direct blood glucose into energy stores.

Conclusion

Dr. Moore's presentation made the surprising case for two distinct intersections between the physiology of drug metabolism and that of diabetes. First, he recounted research that has shown the profound effect that type 1 diabetes can have on the metabolism of certain drugs by stimulating the CAR nuclear receptor. These data may ultimately bear not only on the way type 1 diabetes is treated, but also on the way people with the disease are treated for other conditions. Second, he showed that drug treatments that trigger CAR signaling can impact blood glucose levels in a mouse model of type 2 diabetes, probably by modulating glucose metabolism in the liver. These observations suggest that inhibiting glucose production and/or stimulating glucose storage by the liver is a potentially valuable approach to treating type 2 diabetes, perhaps in conjunction with other therapies.

PATIENT PROFILE

Casey Burkhalter

Using Advanced Technology To Help Control Glucose Levels

It's the middle of the night, and the Burkhalter family of Jacksonville, Florida, is sleeping more soundly and peacefully than they have in a long time because of a newly-developed technology called a continuous glucose monitoring system, or CGMS. This promising device is being tested by their 14-year-old daughter, Casey, as part of a research network, called the Diabetes Research in Children Network (DirecNet), sponsored by the National Institute of Child Health and Human Development, the NIDDK, and the Special Statutory Funding Program for Type 1 Diabetes Research.

Casey has type 1 diabetes. The device she is testing monitors her blood glucose levels almost constantly throughout the day. It's when Casey is asleep at night, however, that the CGMS is a lifesaver for her and her family. Should Casey's glucose levels become too high or too low, the CGMS sets off an alarm that alerts her parents to take action. If her glucose level is too low, the Burkhalters give Casey orange juice to raise her blood glucose levels; if it's too high, they administer insulin through Casey's insulin pump.

Caring for children with diabetes requires great diligence, and CGMS technology has the potential to ease some of that burden. "Prior to Casey using the CGMS," says Casey's mother, Leslie Burkhalter, "my husband and I would wake up every 2 hours to prick Casey's finger and check her glucose levels." Their nightly vigil was part of an all-out effort to keep their daughter's blood glucose levels as close to the normal range as possible to prevent diabetes-related seizures and other complications.

NIDDK-supported research—including the landmark Diabetes Control and Complications Trial (DCCT) and a follow-up study, the Epidemiology of Diabetes Interventions and Complications Study (EDIC)—demonstrated that intensive blood glucose control offers remarkable long-term benefits when it comes to preventing or delaying the damage diabetes can have on a patient's eyes, kidneys, and nerves, as well as the harm the disease can inflict on large blood vessels that can lead to heart attacks and strokes.

Casey and Leslie Burkhalter

"I want to tell the world about this device," says Mrs. Burkhalter.

Most people with diabetes report checking their glucose levels every couple of hours, at best. The CGMS device Casey is testing is designed to provide, among other valuable information, glucose readings every minute—without a finger prick. The hope is that this technology will enable people of all ages with diabetes to better gain control over their blood glucose levels and reduce their risk of diabetes complications.

PATIENT PROFILE

"I want to tell the world about this device," says Mrs. Burkhalter, who works in sales and is also actively involved in the Juvenile Diabetes Research Foundation (JDRF).

About the Continuous Glucose Monitoring System
In 2006, new continuous glucose monitoring devices were approved for use in adults. In 2007, a device was approved for use in children. However, Casey wears her monitor because of her participation in the DirecNet study, which is investigating the potential use of CGMS technology and its impact on the management of type 1 diabetes in children. She received her CGMS when she enrolled in the study in December 2005.

Casey's CGMS comes with a 5-day sensor, a transmitter, and a wireless receiver with a built-in glucose monitoring system. The tiny glucose sensor is placed just under the skin of Casey's abdomen. This procedure is similar to inserting the catheter of an insulin pump, is quick, and usually is not very painful. Tape is used to hold it in place. When used during the day, the wireless receiver allows Casey to attach the monitor to a belt or the waistline of her pants. When she goes to bed, she leaves it on her night table. The system automatically records an average glucose value every minute for up to 5 days, at which time the sensor needs to be replaced and repositioned on Casey's abdomen.

Casey's CGMS, when connected to a computer, also provides charts and graphs that indicate trends in her glucose levels over time and how often her glucose levels may be out of range. Although Casey's blood glucose control was excellent when she entered the trial, while wearing the CGMS, she was able to further improve her blood glucose control, without causing hypoglycemia. The family had great comfort knowing Casey's blood glucose level all the time and in real time.

"This technology is unbelievably helpful in controlling glucose levels. It's a huge step toward an artificial pancreas," says Mrs. Burkhalter. She is referring to the day when glucose monitoring and insulin delivery technologies merge, allowing insulin pumps to not only recommend proper insulin dosages, but automatically deliver them as well.

The Burkhalters' Vigil Before the CGMS
Casey was diagnosed with diabetes at about age 10 and a half. Her 18-year-old brother, Tyler, was diagnosed with the disease in November 1999. "Both came as a surprise," says Mrs. Burkhalter. "There is no other history of diabetes in our family."

Night after night of awaking every 2 hours to check Casey's levels was taking its toll on the family. "It created a lot of wear and tear on my husband and me," says Mrs. Burkhalter. "Lack of sleep was making us both irritable and cranky. However, we didn't want Casey to go into a diabetic seizure in her sleep, and fortunately, she never has."

The CGMS has provided the Burkhalters with more than just a good night's sleep. It has provided their daughter with a new attitude toward managing her diabetes.

Casey, whom her mother describes as outgoing and determined, is also an athlete who plays basketball, rides horses, and is a member of a crew team—all rigorous physical activities that can make controlling glucose levels even more difficult than normal.

"I hate having my fingers pricked (to check glucose levels), and the calluses they make aren't very attractive," says Casey. "With the CGMS, I only need to prick my finger, on average, once instead of 7 to 12 times a day." Casey adds that her CGMS is easy to wear "because it's not technically connected to me." At night, she keeps the transmitter on her nightstand. Should its alarm beep, "my parents don't even need to wake me."

As helpful as it may be, the technology is not perfect. There can be as much as a 10 minute delay between

sensing and reading out glucose levels, and every 5 days, when the sensor needs replacing, it takes 10 hours to recalibrate, both of which timeframes users would like to see shortened, says Mrs. Burkhalter. Making the device smaller would also make it more convenient, she adds.

Participating in DirecNet

The Burkhalters learned about the DirecNet CGMS study when, in the fall of 2005, Mrs. Burkhalter read an article in *Countdown*, a publication of the JDRF, entitled, "Artificial Pancreas: How Close Are We to Closing the Loop?" It piqued her interest, and shortly thereafter she spoke with Casey's endocrinologist, Dr. Nelly Mauras at the Nemours Children's Clinic, one of the five participating centers, who recruited Casey into the study and started the ball rolling. Casey was one of the first 30 children in the U.S. to participate in this DirecNet study. Today there are about 100. "One reason Casey is a good candidate for the study is that she recognizes when her blood glucose level is low during the day but, unlike Tyler, not at night," says Mrs. Burkhalter.

The Burkhalters have been extremely pleased with their participation in the DirecNet study. "We believe in the potential of this technology and very much appreciate how those running the study have provided information to us, as well as taken information from us," says Mrs. Burkhalter. "We've recommended the study to many of our friends, and several of their children are now participants." Tyler, the Burkhalters' son, is not a study participant. "He's averse to wearing anything, including an insulin pump, and unlike, Casey, he doesn't seem to mind the finger pricks as much. But he's beginning to change his mind [about using a CGMS]," says Mrs. Burkhalter.

Five clinical centers participate in DirecNet, including Yale University, The Barbara Davis Diabetes Center (Denver), Nemours Children's Clinic-Jacksonville, University of Iowa, and Stanford University. For more information on participating in DirecNet, please visit: *http://public.direc.net*

PATIENT PROFILE

Modesta Solorzano

Gestational Diabetes Study Focuses on Hispanic Women

Modesta Solorzano was nearly eight months pregnant with her third child when she was recruited for the Gestational Diabetes Mellitus (GDM) Cohort Study. This NIDDK-supported observational study is focused on relatively young Hispanic women who have had gestational, or pregnancy-related, diabetes. The study is helping to identify potential causes of type 2 diabetes in these women, who are at greatly increased risk for the disease because of their history of gestational diabetes.

Modesta Solorzano

At the time she entered the study in 1994, Modesta, like all the original GDM Cohort Study participants, had been diagnosed with GDM. This means that, during her pregnancy, she showed higher than normal levels of glucose (sugar) in her blood after drinking a special high-sugar test drink. Fourteen years later, at age 52, Modesta continues to manifest slightly higher than normal glucose levels when tested, such that she is considered to have "pre-diabetes." However, aided by the study's emphasis on providing information on exercise and healthy diet to participants, she remains free of type 2 diabetes. Also free of diabetes is her son, Anthony, who she proudly says is a "very healthy boy" and straight-A student in school.

About Gestational Diabetes Mellitus (GDM)

GDM is a form of diabetes that is first detected during pregnancy. Like other forms of diabetes, it is characterized by high blood glucose levels and can have serious consequences. Left untreated or uncontrolled, gestational diabetes can result in babies being born very large and with extra fat, which can make delivery difficult and more dangerous for both mother and child. It also can lead to babies with breathing problems and low blood glucose right after birth. Moreover, some studies show that when these babies reach adolescence, they have higher glucose and blood pressure levels and are more likely to be obese than children of women who never had gestational diabetes.

GDM affects about seven percent of all pregnancies in the United States and occurs more frequently among African American, Hispanic/Latina, and American Indian and Alaska Native women, as well as obese women and women with a family history of diabetes. Recent studies also suggest that many or most women diagnosed with GDM already have higher than normal (although not yet diabetic) blood glucose levels before pregnancy, but simply are not tested for this health problem until they become pregnant.

The good news for most women with GDM is that only about 10 percent of them will still have diabetes shortly after their baby is born. The down side is that 20 to 50 percent of them will develop diabetes outside of pregnancy sometime in their life, usually within the next 10 years. Also, they are very likely to have GDM during subsequent pregnancies.

PATIENT PROFILE

GDM affects about seven percent of all pregnancies in the United States and occurs more frequently among African American, Hispanic/Latina, and American Indian and Alaska Native women, as well as obese women and women with a family history of diabetes. Recent studies also suggest that many or most women diagnosed with GDM already have higher than normal (although not yet diabetic) blood glucose levels before pregnancy, but simply are not tested for this health problem until they become pregnant.

Modesta Solorzano came to the United States from El Salvador in 1976. Her family has a long history of type 2 diabetes. Her father died at age 84 of a stroke related to type 2 diabetes. In addition, Modesta's 85-year-old mother suffers from the disease, as do four of her six sisters.

Often, women with gestational diabetes have no symptoms. They also may not know about the link between GDM and the development of type 2 diabetes. Modesta, for example, says that she was unaware of her elevated risk for type 2 diabetes before entering the GDM Cohort Study.

GDM Cohort Study

Specifically targeted to Hispanic women, the University of Southern California (USC) GDM Cohort Study began in 1993. Conducted by a team of researchers led by Dr. Tom Buchanan, it is an observational rather than interventional study—that is, no treatment is involved. During its first several years, the study focused on potential physiological and hormonal factors or problems present in women with GDM that may predict development of type 2 diabetes post-pregnancy. To help identify these factors, the researchers gathered a variety of metabolic and other information from participants. Upon enrollment in the study, women with GDM had three metabolic tests. These tests provided baseline information about factors important in the development of diabetes, such as insulin resistance. Within 6 months after delivery, the participants began a series of regular follow-up visits. Every 15 months, participants had their blood glucose checked, underwent metabolic tests, were given a thorough physical examination, and were asked to fill out a questionnaire related to diet and exercise. They also routinely received advice on diet and exercise that could help them take steps to improve their health and delay or prevent diabetes. These visits have continued into the current, long-term follow up phase of the study. From the beginning, participants have been followed by study investigators until they develop blood sugar levels high enough to require clinical intervention. Women have been followed for as long as 12 to 14 years after their GDM pregnancy.

Fortunately for Modesta, her blood sugar has remained at levels that are relatively easy for her to control through diet and exercise. "I walk a lot," says Modesta, "and I no longer eat a lot of greasy foods." Instead, she has purchased kitchen utensils that enable her to steam vegetables and other foods, and she has reduced her intake of candy and other sweets. "I've lost 12 pounds since being in the study," she says.

Modesta is the mother of three children. Her first child was born in El Salvador 32 years ago. Modesta does not recall having been checked for high glucose levels during that pregnancy. Her second child was born in California 28 years ago. Modesta did not develop GDM as a result of the pregnancy. During her third and final pregnancy, however, Modesta manifested higher than normal glucose levels during a test, and she was therefore recruited to take part in the GDM Cohort Study. She decided to participate in the study because, "I was told that it would help me control my glucose levels and possibly help prevent me from getting type 2 diabetes in the future," she says.

Shortly after enrolling, Modesta gave birth to her third child, Anthony, delivered by Caesarian section. Like many babies of mothers with GDM, Anthony was heavier than normal at time of delivery, weighing just over 9 pounds, but otherwise very healthy.

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Today, at nearly 14 years of age, Anthony remains free of type 2 diabetes even though he is at higher risk for the disease because Modesta experienced GDM during the pregnancy. Modesta makes sure he exercises and eats properly. As a result, Anthony recently lost 15 pounds, going from 140 to 125 pounds. He's a straight-A student, is taking part in his 7th grade decathlon, and has been selected to take special college preparatory classes. "We are very proud of him," says Modesta.

As for the study, Modesta says that she was very happy to be a part of it, was treated very well by the USC study team and learned a lot about her body. For example, she says that she now knows that losing five to seven percent of her normal body weight—even if it means being above ideal weight—is enough to reduce her chances of getting type 2 diabetes. She also learned that she needs to continue to be tested for type 2 diabetes every 1 to 2 years, to help prevent complications—such as heart disease later in life—that could result from progression to type 2 diabetes.

The NIDDK-sponsored GDM Cohort Study ultimately achieved its initial goal of identifying metabolic factors or problems present in Hispanic women during and after a GDM pregnancy that may predict future development of type 2 diabetes. The study team has found that a key predictor of later type 2 diabetes in these women is whether their pancreatic beta cells have difficulty secreting enough insulin to compensate for increased insulin resistance during pregnancy. Importantly, data from this study helped to form the basis for a separate set of studies that have tested interventions to prevent type 2 diabetes in Hispanic women with recent GDM, using medications that can increase sensitivity to insulin and thus reduce stress on the insulin-producing beta cells. Now in a long-term follow-up phase, the GDM Cohort Study is contributing to a better understanding of the chain of events leading to type 2 diabetes in these women who, as a group, are at very high disease risk. This information could also help in designing strategies to identify those individuals at highest risk and reduce their likelihood of developing type 2 diabetes.

"It would be better for members of the Hispanic community if they got more involved in these sorts of programs so that they could learn more about their health," Modesta says.

Modesta says she knows lots of Hispanic women with type 2 diabetes, and has talked about the study to many of her friends. "It would be better for members of the Hispanic community if they got more involved in these sorts of programs so that they could learn more about their health," Modesta says. She adds that studies such as the GDM Cohort Study would "help more Hispanic women take better care of themselves, especially during pregnancy."

Modesta plans to stay in the follow-up portion of the GDM Cohort Study for as long as possible. If there were another study, she'd like to be involved in it, as well. "It's been very helpful to me and my family," she says.

For health information on diabetes, please visit:
http://diabetes.niddk.nih.gov/

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Modesta Solórzano

Estudio de Diabetes Gestacional Centrado en las Mujeres Hispanas

Modesta Solórzano estaba casi en el octavo mes de su tercer embarazo cuando se incorporó al estudio de cohortes sobre la diabetes gestacional. Este estudio de observación está respaldado por el Instituto Nacional de la Diabetes y las Enfermedades Digestivas y del Riñón (NIDDK, por su sigla en inglés) y se centra en mujeres hispanas relativamente jóvenes que han tenido diabetes gestacional, que es la diabetes relacionada con el embarazo. El estudio está ayudando a identificar las posibles causas de diabetes de tipo 2 en estas mujeres, que corren un riesgo mucho mayor de sufrir la enfermedad debido a sus antecedentes de diabetes gestacional.

Modesta Solórzano

Al igual que todas las participantes originales del estudio de cohortes sobre la diabetes gestacional, a Modesta ya le habían diagnosticado diabetes gestacional cuando ingresó en el estudio en 1994. Esto significa que durante su embarazo tuvo concentraciones de glucosa (azúcar) en la sangre más altas que las normales. Esto se descubrió después de una prueba donde tomó una bebida especial con alto contenido de azúcar. Catorce años más tarde, a los 52 años de edad, Modesta sigue teniendo concentraciones de glucosa más altas que las normales cuando se le hacen esta prueba, por lo que se considera que tiene "prediabetes." Sin embargo, gracias al énfasis que hace el estudio en proporcionar a las participantes información sobre el ejercicio y la alimentación saludable, ella aún no ha desarrollado diabetes de tipo 2. Su hijo Anthony tampoco tiene diabetes y Modesta dice con orgullo que "es un chico muy sano" y muy buen estudiante.

Diabetes Gestacional

La diabetes gestacional es una forma de diabetes que se detecta por primera vez durante el embarazo.

En los Estados Unidos la diabetes gestacional afecta a cerca del siete por ciento de todos los embarazos y ocurre con más frecuencia entre las mujeres afroamericanas, hispanas, indígenas americanas y mujeres nativas de Alaska, así como en mujeres obesas y en mujeres con antecedentes familiares de diabetes. Estudios recientes también indican que muchas o la mayoría de las mujeres con diagnóstico de diabetes gestacional ya tienen concentraciones de glucosa en la sangre más altas que las normales antes del embarazo (aunque todavía no sean diabéticas), sino que simplemente no se habían hecho pruebas para este problema de salud hasta que quedaron embarazadas.

Al igual que otras formas de diabetes, se caracteriza por concentraciones altas de glucosa en la sangre y puede tener consecuencias graves. Si no se trata o no se controla, la mujer con diabetes gestacional puede tener un bebé muy grande y con exceso de grasa, lo cual puede dificultar el parto y hacer que

sea más peligroso tanto para la madre como para el hijo. Además, los bebés pueden tener problemas respiratorios y concentraciones bajas de glucosa en la sangre inmediatamente después de nacer. Por otra parte, algunos estudios demuestran que cuando estos bebés llegan a la adolescencia tienen concentraciones más altas de glucosa y tensión arterial más elevada, y son más propensos a la obesidad que los niños de las mujeres que nunca tuvieron diabetes gestacional.

Lo bueno es que sólo un 10 por ciento de las mujeres con diabetes gestacional seguirá teniendo diabetes poco después del parto. Lo malo es que entre un 20 y un 50 por ciento de ellas desarrollará diabetes en algún momento de la vida cuando no estén embarazadas, por lo general en los diez años siguientes. Además, es muy probable que también tengan diabetes gestacional en los embarazos siguientes.

En los Estados Unidos la diabetes gestacional afecta a cerca del siete por ciento de todos los embarazos y ocurre con más frecuencia entre las mujeres afroamericanas, hispanas, indígenas americanas y mujeres nativas de Alaska, así como en mujeres obesas y en mujeres con antecedentes familiares de diabetes. Estudios recientes también indican que muchas o la mayoría de las mujeres con diagnóstico de diabetes gestacional ya tienen concentraciones de glucosa en la sangre más altas que las normales antes del embarazo (aunque todavía no sean diabéticas), sino que simplemente no se habían hecho pruebas para este problema de salud hasta que quedaron embarazadas.

Modesta Solórzano llegó a los Estados Unidos proveniente de El Salvador en 1976 y tiene antecedentes familiares de diabetes de tipo 2. Su padre murió a los 84 años de edad por un derrame cerebral relacionado con la diabetes de tipo 2. Además, la madre de Modesta, que tiene 85 años de edad, es diabética. También son diabéticas cuatro de sus seis hermanas.

A menudo las mujeres con diabetes gestacional no tienen síntomas. Muchas de ellas no saben de la relación que existe entre la diabetes gestacional y la diabetes de tipo 2. Modesta, por ejemplo, dice que antes de ingresar al estudio de cohortes sobre la diabetes gestacional no sabía que corría un riesgo alto de sufrir diabetes de tipo 2.

Estudio de Cohortes sobre la Diabetes Gestacional
La Universidad de Sur de California (USC) estudio de cohortes sobre la diabetes gestacional, dirigido específicamente a mujeres hispanas, comenzó en 1993. Conducida por un equipo de investigadores liderado por el Dr. Tom Buchanan es un estudio de observación más que de intervención; es decir, no se da ningún tratamiento. Durante los primeros años, el estudio se centró en los posibles factores fisiológicos y hormonales o en los problemas presentes en mujeres con diabetes gestacional que pueden predecir la aparición de la diabetes de tipo 2 después del embarazo. Para identificar esos factores los investigadores reunieron muchos datos, tanto metabólicos como de otro tipo, a partir de las participantes. Al ingresar al estudio, se les hicieron tres pruebas metabólicas a las mujeres con diabetes gestacional. Estas pruebas proporcionaron información inicial sobre factores importantes en la aparición de la diabetes, tales como la resistencia a la insulina. Las participantes comenzaron una serie de consultas periódicas de seguimiento dentro de los seis meses siguientes al parto. Cada 15 meses se les determinó la concentración de glucosa en la sangre, se les hicieron pruebas metabólicas, se les hizo una exploración física completa y se les pidió que llenaran un cuestionario relacionado con la alimentación y el ejercicio. Además, periódicamente se les dio consejos sobre la alimentación y el ejercicio, de modo que pudieran tomar medidas para mejorar su salud y así retrasar o prevenir la diabetes. Estas consultas han continuado hasta la fase actual del estudio de seguimiento a largo plazo. Desde el comienzo, a las participantes se les ha hecho seguimiento por investigadores del estudio hasta que presentan

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concentraciones de azúcar en la sangre lo suficientemente altas como para requerir intervención médica. A algunas mujeres se les ha hecho seguimiento hasta 12 a 14 años después de la diabetes gestacional.

Afortunadamente para Modesta, su azúcar en la sangre se ha mantenido en concentraciones que para ella son relativamente fáciles de controlar con la alimentación y el ejercicio. "Camino mucho," dice Modesta, "y ya no como muchos alimentos grasosos." En vez de ello, ha comprado utensilios de cocina que le permiten cocinar al vapor las verduras y otros alimentos, y ha dejado de comer tantos caramelos y otros dulces. Dice que ha bajado 12 libras desde que entró al estudio.

Modesta tiene tres hijos. Tuvo el primero en El Salvador hace 32 años y no recuerda que durante el embarazo le hubieran hecho pruebas de sangre para saber si tenía la glucosa alta. Su segundo hijo nació en California hace 28 años y Modesta no tuvo diabetes gestacional como resultado de ese embarazo. Sin embargo, durante su tercer y último embarazo, Modesta tuvo concentraciones de glucosa en la sangre más altas que las normales durante una prueba y por eso la invitaron a participar en el estudio de cohortes sobre la diabetes gestacional. Dice que decidió participar en el estudio, porque "me dijeron que me ayudaría a controlar las concentraciones de glucosa y que tal vez me ayudaría a prevenir la diabetes de tipo 2 en el futuro."

Poco después de ingresar en el estudio tuvo a su tercer hijo, Anthony, por cesárea. Al igual que muchos bebés de madres con diabetes gestacional, Anthony pesó más de lo normal—un poco más de 9 libras—en el momento del parto, pero aparte de eso era muy sano. Actualmente Anthony tiene casi 14 años de edad y no tiene diabetes de tipo 2, aunque corre un riesgo mayor de sufrir esta enfermedad porque Modesta tuvo diabetes gestacional durante el embarazo. Modesta se asegura de que él haga ejercicio y coma bien. Como resultado, hace poco Anthony también bajó 15 libras: pesaba 140 libras y ahora pesa 125 libras. Es un estudiante sobresaliente de séptimo grado, está participando en el decatlón de su curso y ha sido seleccionado para tomar clases preparatorias especiales para entrar a la universidad. "Estamos muy orgullosos de él," dice Modesta.

En cuanto al estudio, Modesta dice que estaba muy contenta de participar, que el equipo de USC la trataron muy bien y que aprendió mucho sobre su cuerpo. Dice, por ejemplo, que ahora sabe que bajar entre un cinco y un siete por ciento de su peso normal—incluso si esto significa estar por arriba del peso ideal—es suficiente para reducir sus probabilidades de presentar diabetes de tipo 2. También aprendió que necesita seguir haciéndose pruebas para la diabetes de tipo 2 cada año o cada dos años con el fin de prevenir complicaciones en el futuro (por ejemplo, enfermedades del corazón) que podrían presentarse a causa de la progresión a diabetes de tipo 2.

Modesta dice que "sería mejor para los miembros de la comunidad hispana si participaran más en este tipo de programas para que pudieran aprender más sobre su salud."

El estudio de cohortes sobre la diabetes gestacional patrocinado por el NIDDK finalmente logró su objetivo inicial de identificar los factores o problemas metabólicos presentes en las mujeres hispanas durante y después de un embarazo con diabetes gestacional que pueden predecir la aparición de diabetes de tipo 2. El equipo del estudio descubrió en el estudio que un factor pronóstico de la aparición futura de diabetes de tipo 2 en estas mujeres es si las células beta del páncreas no pueden secretar suficiente insulina para compensar el aumento de resistencia a la insulina durante el embarazo. Es importante anotar que los datos de este estudio sirvieron de base para un conjunto distinto de estudios que han evaluado

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intervenciones para prevenir la diabetes de tipo 2 en mujeres hispanas con diabetes gestacional reciente, utilizando medicamentos que pueden aumentar la sensibilidad a la insulina y así reducir el estrés sobre las células beta productoras de insulina. El estudio de cohortes sobre la diabetes gestacional, que ahora se encuentra en una etapa de seguimiento a largo plazo, está contribuyendo a que se entienda mejor la cadena de acontecimientos que causan la diabetes de tipo 2 en estas mujeres que, como grupo, corren un riesgo muy alto de sufrir la enfermedad. Esta información también podría servir para diseñar estrategias con el fin de identificar a las personas que corren el mayor riesgo y reducir sus probabilidades de presentar diabetes de tipo 2.

Modesta dice que conoce a muchas mujeres hispanas con diabetes de tipo 2 y que ha hablado del estudio con muchos de sus amigos. También dice que "sería mejor para los miembros de la comunidad hispana si participaran más en este tipo de programas para que pudieran aprender más sobre su salud." Agrega que estudios tales como el estudio de cohortes sobre la diabetes gestacional "ayudarían a más mujeres hispanas a cuidarse mejor, especialmente durante el embarazo."

Modesta piensa permanecer el mayor tiempo posible en la parte de seguimiento del estudio de cohortes. Si hubiera otro estudio, también le gustaría participar. Dice que "ha sido muy útil para mí y para mi familia."

Para más información acerca de diabetes, vea: *www.diabetes-espanol.niddk.nih.gov*

The National Diabetes Education Program: It's Never Too Early To Prevent Diabetes

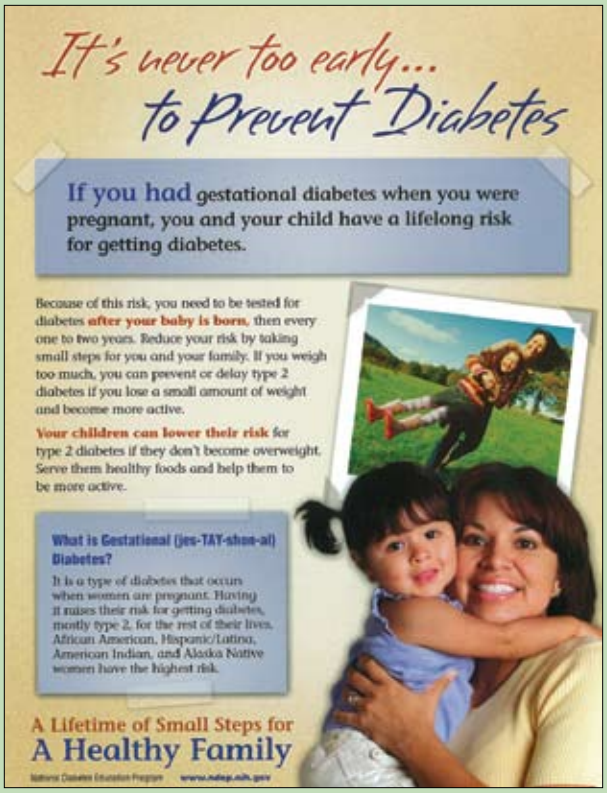
Gestational diabetes mellitus (GDM) is a form of glucose intolerance that is diagnosed during pregnancy. GDM affects about 7 percent of all U.S. pregnancies annually, resulting in approximately 200,000 cases a year.[1] Women diagnosed with GDM not only face an increased risk of complications during pregnancy and delivery, but they and their children bear an elevated risk of developing type 2 diabetes in the future. An information campaign developed by the National Diabetes Education Program is raising awareness of this risk and of ways to reduce its impact.

Who is at risk? Gestational diabetes occurs more frequently among obese women and women with a family history of diabetes, and among African American, Hispanic/Latina, and American Indian and Alaska Native women. Women who have had GDM have an increased risk of GDM in a subsequent pregnancy.

After pregnancy, 5 to 10 percent of women who had GDM are found to have type 2 diabetes. Women who have had GDM have a 20 to 50 percent chance of developing diabetes in the next 5 to 10 years following pregnancy. The children of women with a history of GDM are at an increased risk for obesity and diabetes compared to other children.

Recent reports have shown high or increasing rates for GDM in various parts of the country, including:[1]

- Washington, D.C, where, in 2003, the GDM prevalence rate in Hispanic women was 12 percent —close to the highest rate of 14 percent seen in some American Indian women.
- New York City, where the GDM prevalence rate increased 46 percent from 1990 to 2002—with the highest increase found among Asian women.
- Colorado, where the GDM prevalence rate increased 95 percent from 1994 to 2002— with the highest increase among Hispanic women.
- Northern California, where the number of new cases each year increased 35 percent from 1991 to 2000.



The educational campaign, "It's Never too Early To Prevent Diabetes," is spreading the word about the risk for type 2 diabetes faced by women with a history of gestational diabetes mellitus and their children.

These regional GDM prevalence rates raise concern that the increase may reflect the ongoing pattern of increasing obesity and contribute to the upsurge in cases of diabetes in the U.S.

The latest diabetes prevention campaign message by the National Diabetes Education Program (NDEP) is: *It's Never Too Early To Prevent Diabetes: A Lifetime of Small Steps for a Healthy Family.* This campaign, which was launched by Dr. Griffin Rodgers, Director, NIDDK, together with then-Deputy Surgeon General Kenneth Moritsugu, is spreading the word about the risk for type 2 diabetes faced by women with a history of gestational diabetes mellitus and their offspring. The message, *It's Never Too*

Early To Prevent Diabetes, is the latest addition to NDEP's campaign, *Small Steps. Big Rewards*. *Prevent Type 2 Diabetes*, the nation's first comprehensive multicultural type 2 diabetes prevention campaign. The campaign offers materials that can help women with a history of gestational diabetes take steps to prevent or delay type 2 diabetes and help their children lower their risk for the disease.

To ensure that the campaign effectively reaches the broad audience of women and children at risk, materials include a tip sheet in both English and Spanish for women who have had GDM, a tip sheet in English and Spanish for children at risk for type 2 diabetes, and a booklet for adults to help women and their families make healthy food choices and be more physically active to prevent or delay type 2 diabetes.

The campaign is based in large part on results from the Diabetes Prevention Program (DPP) clinical trial. This NIDDK-funded study found that people at increased risk for type 2 diabetes can prevent or delay the onset of the disease by losing 5 to 7 percent of their body weight through increased physical activity and a low-fat, low-calorie eating plan. The DPP included several hundred women with a history of gestational diabetes, and the powerful reduction in risk of diabetes demonstrated in the study—up to 58 percent—was found in all subgroups, including this group of women.

The U.S. Department of Health and Human Services' National Diabetes Education Program is jointly sponsored by the National Institutes of Health and the Centers for Disease Control and Prevention with the support of more than 200 partner organizations. The NDEP has materials for health care professionals and people at risk for diabetes—including older adults, American Indians and Alaska Natives, Hispanics/Latinos, African Americans, and Asian Americans and Pacific Islanders. For more information about the NDEP or to obtain a copy of the new It's Never Too Early To Prevent Diabetes *and* Nunca es muy temprano para prevenir la diabetes *tip sheets and other* Small Steps. Big Rewards. *diabetes prevention materials, visit www.ndep.nih.gov*

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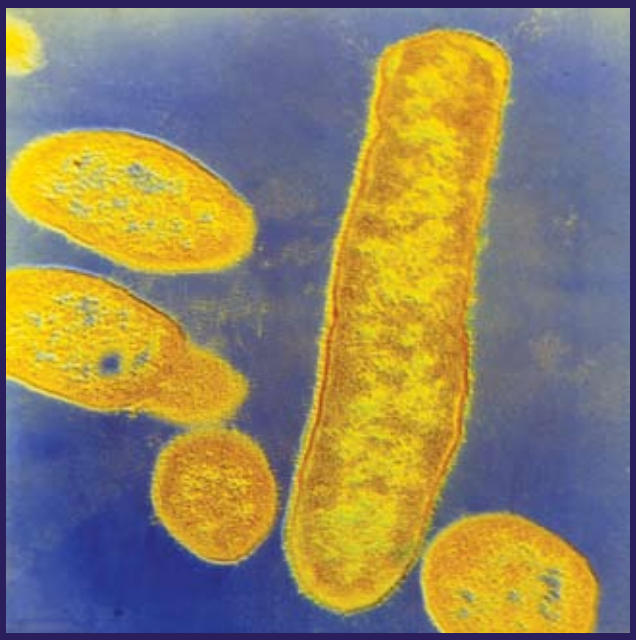
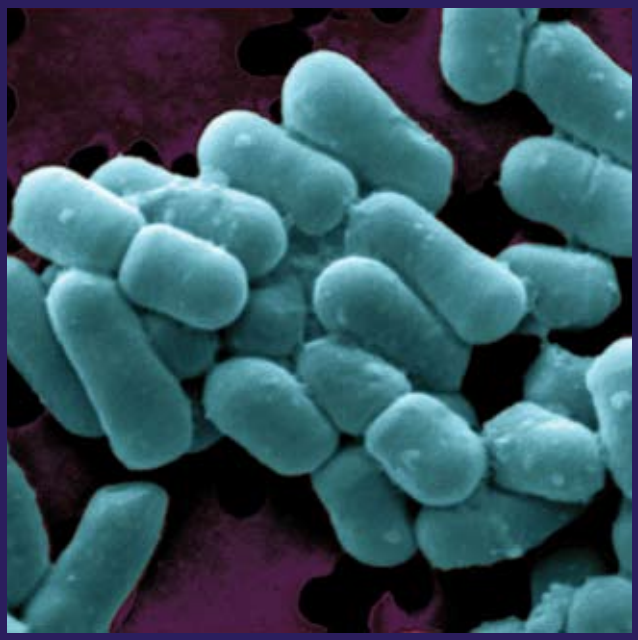
[1] *www.nih.gov/news/pr/apr2006/niddk-25.htm*





In addition to genetics and lifestyle, researchers supported by the NIDDK have found an important "insider" that also contributes to human obesity: the trillions of bacteria and other tiny organisms (microbes) residing in the human gut. Recent studies detailed in this chapter show the potent effects that certain bacteria have on their host's nutrient absorption and metabolism, which contribute to obesity. For example, researchers have discovered that obese and lean individuals differ in their relative abundance of intestinal bacteria with different energy-harvesting abilities from the Bacteroidetes (top image) and Firmicutes (bottom image) bacterial divisions.

Top image credit: CNRI/Photo Researchers Inc.

Bottom image credit: Scimat/Photo Researchers Inc.

Obesity

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

Approximately one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2,3] Furthermore, while obesity and overweight have risen in the population in general, the greatest increases observed over approximately the past two decades have been in the prevalence of extreme obesity; those who are severely obese are most at risk for serious health problems.[4] Levels of childhood overweight have also escalated in the past several decades; approximately 17 percent of children and teens ages 6 through 19 are now overweight.[1,2,5,6] The levels of pediatric overweight have ominous implications for the development of serious diseases both during youth and later in adulthood. Overweight and obesity also disproportionately affect racial and ethnic minority populations, and those of lower socio-economic status.

The increased prevalence of obesity in the U.S. is thought to result from the interaction of genetic susceptibility with behavior and factors in the environment that promote increased caloric intake and sedentary lifestyles. Thus, the NIDDK supports a multidimensional research portfolio on obesity, ranging from basic studies to large clinical trials. Examples include: investigations to elucidate the hormones and other signaling molecules that influence appetite and energy expenditure, and that link obesity to type 2 diabetes and other adverse health conditions; research on the role of inflammation in obesity-associated health problems; studies of the role of gut bacteria in obesity; exploration of genetic factors that predispose individuals to obesity; research on nutrition and physical activity; the development and testing of modifications to environmental factors in schools, the home, and other settings as strategies for obesity prevention; and research on bariatric surgery as a treatment for severe obesity. The NIDDK additionally supports studies of eating disorders that are associated with obesity in some people.

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

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

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

[3] National Center for Health Statistics. Obesity Among Adults in the United States—No Significant Change Since 2003-2004. Data Brief Number 1. Hyattsville, MD: Public Health Service. 2007.

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

[5] National Center for Health Statistics. Chartbook on Trends in the Health of Americans. Health, United States, 2006. Hyattsville, MD: Public Health Service. 2006.

[6] This document uses the terms overweight and obesity interchangeably for children and adolescents because there is no generally accepted definition for obesity, as distinct from overweight, in this age group.

supporting a spectrum of research studies consistent with the recommendations of the *Strategic Plan.*

WEIGHT REDUCTION IN INDIVIDUALS WITH TYPE 2 DIABETES

First Year of the Look AHEAD Trial Yields Encouraging News for Patients with Type 2 Diabetes: After 1 year, individuals in the Look AHEAD (Action for Health in Diabetes) clinical trial who were assigned to an intensive lifestyle intervention had significantly greater improvement in health measures than did individuals receiving diabetes support and education alone. The Look AHEAD trial enrolled over 5,000 patients to determine whether the intensive lifestyle intervention could impact the long-term health of overweight and obese adults with type 2 diabetes. Half of the patients received this intervention, which consisted of weight loss through decreased calorie consumption and increased physical activity. This group of patients also received counseling through weekly individual or group meetings with a team of specialists. The diabetes support and education intervention consisted of three diabetes education sessions held throughout the year. Assessments of the use of diabetes, blood pressure, and cholesterol medication; weight; blood sugar (glucose) levels; blood pressure; cholesterol levels; kidney function; presence of the metabolic syndrome; and other measures were completed for all patients at the beginning of the trial and after 1 year of the interventions. The Look AHEAD trial was designed to follow the participants for up to 11.5 years, if specific criteria during the first year were met. These included a greater than 5 percent difference in average weight change between the two intervention groups and a greater than 5 percent loss in average weight over 1 year in the intensive lifestyle intervention group. Both of these criteria were exceeded. In addition to the greater weight loss in the patients receiving the intensive lifestyle intervention, these individuals also had lower blood glucose levels; decreased use of medications for diabetes, blood pressure, and cholesterol; and improved cholesterol and blood pressure levels. As the Look AHEAD trial continues over the next several years, it will provide valuable information regarding the use of an intensive lifestyle intervention for reaching and maintaining a weight loss goal in people with type 2 diabetes, and whether the promising results seen thus far lead to long-term health benefits associated with weight loss.

Look AHEAD Research Group, Pi-Sunyer X, Blackburn G, Brancati FL, Bray GA, Bright R, Clark JM, Curtis JM, Espeland MA, Foreyt JP, Graves K, Haffner SM, Harrison B, Hill JO, Horton ES, Jakicic J, Jeffery RW, Johnson KC, Kahn S, Kelley DE, Kitabchi AE, Knowler WC, Lewis CE, Maschak-Carey BJ, Montgomery B, Nathan DM, Patricio J, Peters A, Redmon JB, Reeves RS, Ryan DH, Safford M, Van Dorsten B, Wadden TA, Wagenknecht L, Wesche-Thobaben J, Wing RR, and Yanovski SZ: Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: One-year results of the Look AHEAD trial. Diabetes Care 30: 1374-1383, 2007.

GUT BACTERIA AND OBESITY

New Insights on the Relationship Between Obesity and Gut Bacteria: While genetics and lifestyle can conspire to promote obesity, an additional potential accomplice has emerged from recent research: the bacteria and other tiny organisms (microbes) that normally reside in the gut. Studies are revealing how some of these trillions of microbes may not only contribute to a flood of extra calories, but also modulate the biologic pathways that regulate metabolism and whether calories are burned or stored as fat.

Able to digest many dietary components that the body's own intestinal cells can't, gut microbes offer extra energy from food in exchange for a home in the gut. However, some may provide more food energy than others in the form of calories. By analyzing the genomes of these microbes, referred to collectively as the "microbiome," scientists discovered that the relative abundance of two major types of gut bacteria differ between lean and obese mice. They additionally found, through genomic and biochemical analyses, that these types of gut bacteria differ in their capacity to harvest energy from food. Those more prevalent in the obese mice are better able to extract calories—and potentially provide too many extra calories to their mouse "hosts." In a parallel study in humans, the scientists found that the relative abundance of these types of gut bacteria also differs between lean and obese people. Additionally, when the obese study volunteers lost weight by dieting, the relative proportions of these bacteria in their guts also changed. To further explore these effects, the researchers turned again to mice. They raised several

mice in "germ-free" conditions so the mice would lack normal gut bacteria and then gave them gut bacteria from other (donor) mice. No longer germ-free, the mice who received gut bacteria from obese donors gained significantly more body fat over the next two weeks than mice who received gut bacteria from lean donors.

In another set of experiments in mice, scientists found that the bacteria that live in the gut also engage in a form of home remodeling, at the molecular level. They reduce the amounts of some native mouse proteins that would otherwise keep body weight down. The researchers first found that germ-free mice do not gain as much weight on a high-fat "Western diet" as do mice that have normal gut bacteria. They then showed that mice raised germ-free have increased activity of a protein called AMPK, which helps burn fat in muscles and the liver. Building on previous research, they found that gut microbes may also contribute to diet-induced obesity by reducing levels of another mouse protein, called Fiaf, as well as mouse proteins that are regulated by Fiaf, which results in enhanced fat storage. Finally, using an implantable device in the mice to track locomotion, the scientists observed that mice raised without gut microbes move more, and thus may be burning more calories than mice with gut bacteria.

These studies show that the gut's resident microbes affect not only the amount of calories obtained from food, but also whether the calories are stored or burned. Manipulation of the composition of gut bacteria may one day be a novel approach to obesity prevention or treatment.

Bäckhed F, Manchester JK, Semenkovich CF, and Gordon JI: Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA 104: 979-984, 2007.

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, and Gordon JI: An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444: 1027-1031, 2006.

MOLECULAR CONTRIBUTORS TO OBESITY

A High-Fat Diet, Inflammation, and Metabolic Problems—New Insights: As scientists seek to understand how obesity causes chronic and devastating health problems, they have uncovered a convergence between inflammation and impaired response to the hormone insulin. Obesity can hinder the ability of tissues throughout the body to respond properly to insulin. Such insulin resistance is a harbinger of serious disease, including type 2 diabetes. In addition to obesity and insulin resistance, a high-fat diet has also been associated with inflammation. Recent studies are illuminating how fat co-opts the immune system to produce inflammation by affecting cells called macrophages and other inflammatory factors.

To gain insight into how fat triggers inflammation, and consequently insulin resistance, one research group sought clues from a different biological process: fighting infection. Many bacteria are coated with a fat-containing substance which activates inflammatory pathways, specifically those controlled by a protein called TLR4. Perhaps, then, fatty acids from food might also incite inflammation via TLR4. To test this hypothesis, the scientists began by comparing macrophages and fat cells from normal mice to those from mice lacking TLR4 (due to genetic mutation). They found that fatty acids induced molecular changes associated with inflammation in both the macrophages and fat cells from normal mice, but not in those lacking TLR4. These findings demonstrated the need for TLR4 in this process. Bringing obesity into the picture, the scientists then discovered that there is more TLR4 in fat tissue from obese mice than from lean mice. Exploring the effects of dietary fat, they found that nutritional fatty acids, when injected into mice, elicited signs of inflammation and insulin resistance, but these were reduced in mice lacking TLR4. Further experiments in the mice also suggested that TLR4 may play a role in the metabolic problems associated with a high-fat diet. Thus, the investigators discovered that a protein known for its beneficial function in fighting bacterial infection, TLR4, may also play an adverse role in metabolism as a link among fat, inflammation, and insulin resistance.

The focus of another group of scientists was a protein called Cap, which earlier studies had suggested may play a role in insulin action. To better understand this protein, the scientists generated mice that lacked the Cap protein, and fed them a high-fat diet. In normal mice, a high-fat diet impedes the ability of muscle, liver, and fat tissue to respond properly to insulin. Muscles do not take up glucose sufficiently from the bloodstream; the liver continues producing excess glucose; and fat

tissue is impaired in its processing of fatty acids—resulting in too much fat in the blood. In mice lacking the Cap protein, however, measures of glucose uptake and production, fatty acids in blood, and other factors indicated protection from insulin resistance. This result was surprising and intriguing, in light of earlier studies of the Cap protein. Pursuing a potential role for Cap in the inflammation associated with insulin resistance, the investigators further examined mice fed a high-fat diet. They observed that many fewer macrophages had infiltrated the fat tissue of Cap-deficient mice, as compared with normal mice. Additional experiments showed that isolated macrophages, normally mobile, are less able to migrate when deficient in Cap protein. Finally, the investigators generated mice that lacked Cap protein only in their macrophages and other immune cells, but not elsewhere in the body. They did this by transplanting bone marrow from Cap-deficient mice into normal mice whose own immune systems had been eradicated. (Although other types of immune cells derive from bone marrow, the scientists pointed out that only macrophages are thus far known to play a role in insulin resistance.) The transplanted macrophages, absent the Cap protein, were able to protect the normal mice from insulin resistance induced by a high-fat diet. Thus, the Cap protein is used in macrophages to modulate inflammation as an unhealthy response to a high-fat diet.

These studies shed new light on the pathways leading from a high-fat diet and obesity to inflammation and then to insulin resistance. Additionally, they open new avenues for further research into the complex biological origins of obesity-related metabolic disease, which may inform the development of new therapeutic approaches.

Lesniewski LA, Hosch SE, Neels JG, de Luca C, Pashmforoush M, Lumeng CN, Chiang SH, Scadeng M, Saltiel AR, and Olefsky JM: Bone marrow-specific Cap gene deletion protects against high-fat diet-induced insulin resistance. Nat Med 13: 455-462, 2007.

Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, and Flier JS: TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116: 3015-3025, 2006.

Blood Protein Warns of Hidden Belly Fat and Disease Risk: People with excess deep-belly fat are known to be at increased risk for cardiovascular disease and type 2 diabetes. Now scientists have found that this type of fat, compared to other types, produces higher levels of a protein that can be detected in the blood. The protein may serve as a simple indicator for deep visceral fat and disease risk. In recent years, scientists supported by the NIDDK have been exploring the unexpected complexity of the molecule retinol-binding protein 4 (RBP4), once thought to have the sole purpose of ferrying vitamin A (retinol) through the bloodstream. The researchers showed that it appears to affect insulin resistance, a risk factor for diabetes, in mice. In people, RBP4 is elevated in obese or diabetic individuals with insulin resistance, and it drops with exercise training or other interventions known to reverse insulin resistance.

In the new study, the scientists set out to determine if blood levels of RBP4 were directly influenced by visceral fat, which surrounds abdominal organs and has been linked to disease risk. They measured blood levels of RBP4 in 130 obese and 66 lean people. Each participant underwent a computed tomography (CT) scan to assess relative amounts of visceral fat and subcutaneous fat, which lies just beneath the skin. The researchers also analyzed RBP4 gene expression in small samples of both visceral and subcutaneous fat from each person. The scientists found that, overall, RBP4 gene expression was 5-fold higher in visceral than in subcutaneous fat. In obese people with a "visceral fat" pattern of obesity, RBP4 gene expression in visceral fat cells was 60-fold higher than in the lean group. By comparison, RBP4 expression was just 12-fold above normal in people with a "subcutaneous fat" pattern. Blood levels of RBP4 measured up to 3 times higher in obese than in lean people. Those with higher blood levels of RBP4 had more abdominal fat and lower insulin sensitivity, regardless of their age, gender or body mass index (a ratio of weight to height). Among several fat-secreted proteins now associated with insulin activity, blood concentration of RBP4 is thus far the strongest predictor of a person's visceral fat load and insulin resistance. With further study, RBP4 may serve as a convenient marker to identify patients at risk for type 2 diabetes and cardiovascular disease.

Klöting N, Graham TE, Berndt J, Kralisch S, Kovacs P, Wason CJ, Fasshauer M, Schön MR, Stumvoll M, Blüher M, and Kahn BB: Serum Retinol-Binding Protein is more highly expressed in

visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. Cell Metab 6: 79-87, 2007.

—Reprinted, in slightly modified form, from NIH Research Matters; original article by Vicki Contie published July 23, 2007.

Extending Lifespan—Effects of Insulin Signaling and the IRS2 Protein in the Brain: Recent studies are illuminating the role of a protein called IRS2 in coordinating calorie intake, physical activity, and antioxidant function to affect lifespan. This protein had been known to work with the hormone insulin in helping the body to maintain normal blood sugar levels and in modulating other aspects of metabolism. (Its name is an acronym for an "insulin receptor substrate.") In earlier experiments, researchers had found that mice completely lacking IRS2 developed high blood sugar levels, which progressed to severe type 2 diabetes at a relatively young age. These mice also had reduced brain growth. Recently, scientists examined mice that had lower-than-normal levels of IRS2 due to a genetic mutation, but were not completely IRS2-deficient. Paradoxically, the scientists found that the lifespan of these mice was 17 percent longer than their normal counterparts. Delving further into the role of IRS2 in longevity, the researchers focused on the effects of this protein in the brains of mice. Using genetic engineering, they generated mice with lower levels of IRS2 in their brains, but normal levels elsewhere in the body. These mice also had significantly longer life spans than normal mice. Thus, although some IRS2 protein in the body is necessary for health, as evident from the earlier experiments, there can be too much of a good thing: reducing signaling through the IRS2 protein in mice increases their lifespans.

In experiments to better understand this phenomenon, the scientists assessed various metabolic and other health-related factors in the mice. Among their findings, they discovered that older mice with reduced IRS2 in their brains were about twice as active as those with levels of this protein that were closer to normal. Additionally, a measure of glucose metabolism in these mice appeared to be more in the healthy range. The scientists also explored another area commonly associated with aging—antioxidants. The body produces enzymes that have antioxidant activity to help protect cells. Because reduced insulin-like signaling has been shown to increase production of these enzymes, the scientists examined a key antioxidant enzyme in the brains of their mice. Mice with reduced levels of IRS2 in their brains had more of the protective antioxidant enzyme in old age. Based on this research, the scientists suggest a potential implication for human aging. Perhaps human longevity could be extended through strategies to decrease the requirement for IRS2 signaling. Such efforts could include exercise and reduced calorie intake, which would lower the amount of insulin and IRS2 needed to process sugar from food. Interestingly, calorie restriction is associated with longevity in animals. This study thus sheds new light on the connections between aging and metabolism.

Taguchi A, Wartschow LM, and White MF: Brain IRS2 signaling coordinates life span and nutrient homeostasis. Science 317: 369-372, 2007.

Compound Improves Fitness and Survival in Overweight Mice: Two groups of researchers have shown that a naturally-occurring compound called resveratrol significantly improves health and lifespan in overfed mice. Obesity is linked to a host of health problems including type 2 diabetes and cardiovascular disease, and reduced life expectancy. A high-fat diet causes weight gain and insulin resistance in mice compared to animals eating a standard diet. As in humans, these developments shorten lifespan. The researchers found that resveratrol prevented insulin resistance in mice on a high-fat diet, and increased lifespan almost to that of control mice fed healthier food. Although one group found that resveratrol helped prevent weight gain in the mice, the other did not see a significant difference. Both groups also found that measures of physical fitness, such as motor coordination and stamina, were improved in the mice given resveratrol. Although it is not yet known whether resveratrol will have a similar effect in humans, these results suggest the possibility that a dietary supplement may one day help prevent some of the health problems associated with a high-fat/high-calorie diet.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, and Auwerx J: Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127: 1109-1122, 2006.

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, and Sinclair DA: Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444: 337-342, 2006.

DETERMINANTS OF FAT FORMATION

Insights into How Fat Is Stored in the Body: Three recent studies have examined how much fat is stored in the body, what type of fat tissue is made, and where in the body the fat is stored. The findings may provide new pathways to target for therapies.

One group of researchers studied a gene, *adipose*, whose mutation led to increased fat formation in several experimental organisms. Conversely, when the researchers genetically-engineered mice to have extra copies of the *adipose* gene, and thus likely increased amounts of the protein it encodes, they observed a reduction in the storage of fat. This "adipose" protein interacts with other proteins that are involved in turning genes off, suggesting that it may inhibit fat formation by turning off genes that would promote the storage and maintenance of fat.

Another group of researchers, studying mice, identified a gene, *PRDM16*, which plays a major role in directing the development of brown fat cells. Fat cells are of two types: white fat cells, which store energy (as fat), and brown fat cells, which release energy in the form of heat. The researchers showed that expression of *PRDM16* turned on genes and activities that are characteristic of brown fat cells. Like the protein made from the *adipose* gene, the PRDM16 protein also has its effect on fat by regulating whether genes are turned on or off. Adult humans do not have much brown fat naturally, but this study of PRDM16 could have therapeutic implications as it may be possible to stimulate the formation of brown fat cells to release energy as heat, thus preventing excess fat accumulation.

In the final study, researchers genetically modified obese mice to alter where fat is stored in the body. They began with mice that are obese as a result of deficiency in the hormone leptin. In these mice, fat is stored not only in fat tissue, but also in non-fat tissues like the liver and muscle, which may lead to a number of the health problems seen in these mice, such as insulin resistance, a condition associated with type 2 diabetes. When the researchers engineered these mice to produce higher amounts of a protein called adiponectin, the excess fat was no longer stored in non-fat tissues such as the liver, but rather in fat tissue beneath the skin, resulting in even greater obesity. Despite this excess fat tissue, the mice had normal glucose and insulin levels and did not have many of the disease symptoms and morbidity associated with obesity and type 2 diabetes. This result suggests that the location of fat storage is important in how the body responds to obesity. This mouse model may be useful for further research to understand fat storage and its relation to metabolic disease.

Each of these studies contributes more knowledge to our understanding of how fat is stored in the body. This research also reveals potential new areas for the development of therapeutics to combat obesity.

Suh JM, Zeve D, McKay R, Seo J, Salo Z, Li R, Wang M, and Graff JM: Adipose is a conserved dosage-sensitive antiobesity gene. Cell Metab 6: 195-207, 2007.

Seale P, Kajimura S, Yane W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, and Spiegelman BM: Transcriptional control of brown fat determination by PRDM16. Cell Metab 6: 38-54, 2007.

Kim J-Y, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ, and Scherer PE: Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest 117: 2621-2637, 2007.

RESEARCH ON GASTRIC BYPASS SURGERY

Gastric Bypass Surgery Improves Longevity for the Severely Obese: Obesity places an individual at increased risk for type 2 diabetes, cancer, cardiovascular disease, and many other serious health problems. The popularity of diet books is evidence that, for many Americans, gaining weight seems to be very easy; however, losing those extra

pounds is difficult, and keeping them off can be a daunting task. Achieving and maintaining a healthier weight has not been an attainable goal for many who are morbidly obese. A procedure called gastric bypass surgery (a form of bariatric surgery) appears to be a successful solution for some of these individuals. Although gastric bypass surgery helps severely obese patients lose weight, it is an invasive surgery that involves risk. A recent NIDDK-supported research study, however, determined that gastric bypass surgery allows severely obese individuals the opportunity to enjoy longer, healthier lives.

The retrospective study to determine the long-term mortality after gastric bypass surgery was conducted with a cohort of 7,925 severely obese adult patients who had undergone gastric bypass surgery. Also participating in the study was a control cohort of 7,925 severely obese individuals who had not undergone gastric bypass surgery. Both groups were closely matched for age, sex, and body mass index. Scientists used the National Death Index to determine the death rates of members of both groups from all causes, as well as the death rates from specific causes. Notably, deaths by all causes were reduced 40 percent in the group who had surgery. When the numbers of deaths by specific causes were compared, it was found that in the surgery group, deaths caused by diabetes were reduced 92 percent, deaths from coronary artery disease were reduced by 56 percent, and deaths from cancer were decreased by 60 percent. Surprisingly, deaths that were not caused by disease, such as accidents and suicide, increased by 58 percent in the group who had undergone gastric bypass surgery. This increase in non-disease-related deaths warrants additional research.

This study provides much needed information for clinicians and patients considering gastric bypass surgery. Although gastric bypass surgery cannot be considered the optimal treatment for all obese patients, for some with extreme obesity it is currently the most effective treatment available. Previous studies have shown that quality of life improves for obese patients who undergo gastric bypass surgery. This study suggests that undergoing gastric bypass surgery may also decrease the risk of death from diabetes, cardiovascular disease, and cancer.

Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, LaMonte MJ, Stroup AM, and Hunt SC: Long-term mortality after gastric bypass surgery. N Engl J Med 357: 753-761, 2007.

New Metabolic Clinical Research Unit

On January 25, 2007, the NIDDK, in collaboration with the NIH Clinical Center, opened the new NIH Metabolic Clinical Research Unit (MCRU). Located in the NIH Clinical Center, the unit houses facilities that are permitting investigators to conduct cutting-edge research on the physiology, prevention, and treatment of obesity.

Obesity's "connection to co-morbid conditions like diabetes, heart disease and some forms of cancer will drive public health in the future," said NIH Director Dr. Elias Zerhouni, adding that the NIH Clinical Center is the ideal facility for multi-disciplinary research required to address the obesity epidemic.

A component of the *Strategic Plan for NIH Obesity Research*, the unit is designed to foster a collaborative research approach, bringing together experts from the fields of metabolism, endocrinology, nutrition, cardiovascular biology, gastroenterology, hepatology, genetics and the behavioral sciences.

"This is a unique facility that will house research protocols from several of the Institutes, making it the home of trans-NIH research at the Clinical Center," said Dr. Griffin Rodgers, NIDDK Director and co-chair of the NIH Obesity Research Task Force.

The metabolic unit includes 10 private inpatient rooms, a metabolic kitchen, an exercise room, special vending machines, and a communal dining area. The design of each room took into account the needs of the patient volunteers, with specially reinforced construction, amenities and equipment. The metabolic kitchen allows dietitians to control and analyze the composition of the patients' meals to calculate the exact nutrients consumed.

The unit's exercise equipment, physical activity monitors and body composition measurement tools are key resources for research protocols. The fitness equipment, including a treadmill and stationary upright and recumbent bikes, allows researchers to conduct stress and pulmonary function tests to observe the effects of exercise on weight loss.

To measure body composition, the unit provides access to a "Bod Pod®" and DXA scanner. The Pod measures total body density and lean and fat body mass using air displacement. The scanner sweeps the entire body with a small-dose X-ray to calculate how much of the body is made up of fat, muscle, and bone.

The signature feature of the metabolic unit is three "rapid response respiratory suites." These rooms allow researchers to measure volunteers' energy metabolism over 24 hours using non-invasive means. By analyzing air composition in the suite, researchers will be able to determine how much energy on a minute-to-minute basis a volunteer burns while sleeping, eating, or exercising and whether the energy comes from carbohydrate, protein, or fat. The metabolic suites also feature custom-designed vacuum-sealed portholes, or "isolette systems," through which measured food and other items can be passed and blood samples can be taken. These portholes also ensure that physiological measurements are not disrupted.

"People become obese or overweight because of small differences between calories taken in and calories expended over the long term," said Dr. Monica Skarulis, a senior clinical investigator with NIDDK's Clinical Endocrine Branch, who was integral to the planning of the metabolic suites. "These suites will allow clinical researchers to collect precise and accurate measurements necessary to test new and innovative hypotheses about energy metabolism."

The unit, which is available to all NIH Institutes and Centers for obesity research, opened with protocols sponsored by several Institutes including the NIDDK and the National Institute of Child Health and Human Development. The protocols will address how factors, such as a person's diet, level of exercise, or the amount of sleep he or she gets, combine with genetics to determine body weight. Dr. Skarulis said the unit seeks both obese and non-obese volunteers, "so everyone is welcome to consider enrolling."

For more information or to take a video tour of the new Metabolic Clinical Research Unit, please visit: *www2.niddk.nih.gov/Research/ClinicalResearch/MCRU/*

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We Can!: *A National Program on Ways to Enhance Children's Activity & Nutrition*

To help address the growing problem of overweight in American children, the NIDDK is taking part in a national public education program called ***We Can!***, short for "Ways to Enhance Children's Activity & Nutrition." This program is a collaboration of the National Heart, Lung, and Blood Institute with the NIDDK, National Institute of Child Health and Human Development, and National Cancer Institute.

Overweight is a major public health concern in the United States that increasingly affects the nation's children and teens. Young people who are overweight are at greater risk for a lifetime of serious health problems, such as type 2 diabetes, high blood pressure, high blood cholesterol, heart disease, and asthma. The ***We Can!*** program was designed to help children ages 8-13 achieve and maintain a healthy weight, with support from their families and communities. Research-based program materials provide parents and caregivers with tips and activities to encourage three important behaviors to address childhood obesity:

- Improving food choices;
- Increasing physical activity; and
- Reducing time in front of the TV, video game, and computer screens.

We Can! is engaging with many professional, corporate, and community partners to reinforce its message.

As of December 2007, the ***We Can!*** program had grown from 14 intensive community sites to over 450 communities across 44 states since its launch in June 2005. The program also has a global reach, with sites located in Puerto Rico, Canada, Nigeria, the Northern Mariana Islands, and the Philippines. ***We Can!*** community sites include schools, parks and recreation departments, YMCAs, hospitals, health systems, universities, worksites, faith-based organizations, museums, and other settings. In April 2007, the NIH launched the ***We Can!*** City Program, with South Bend, Indiana; Gary, Indiana; and Roswell, Georgia becoming the first three cities to join the program. Since that time, other cities designated as ***We Can!*** Cities include Carson City, Nevada; Las Vegas; Boston; and Pittsburgh. In September, the first ***We Can!*** County joined the program representing Armstrong County, Pennsylvania.

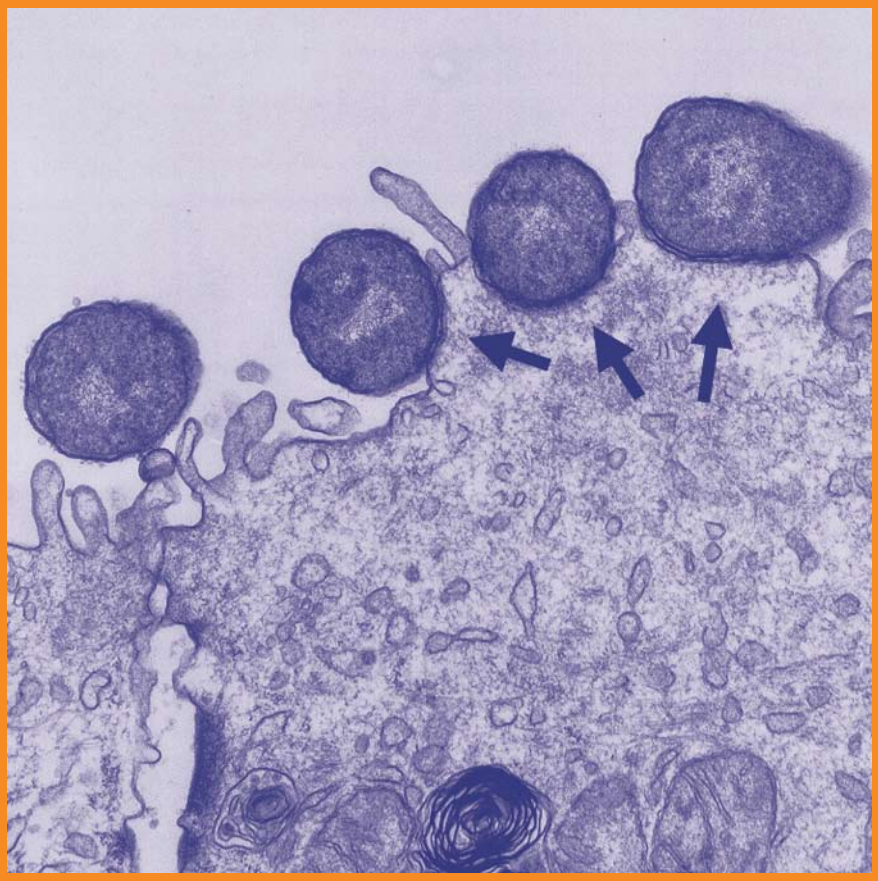
These organizations, cities, and counties are conducting community-based programs and activities for parents/caregivers and children that engage multiple partners, including the media, in focusing on the need to prevent childhood obesity. City and county employees are also encouraged through ***We Can!*** programs and materials to maintain a healthy weight. The NIDDK Director, Dr. Griffin Rodgers, participated in launching a kick-off event in Roswell, Georgia, at which he presented Mayor Jere Wood with a road sign naming Roswell as an official ***We Can!*** City.

"The alarming trend of overweight in our children puts their health and well-being at risk," said Dr. Rodgers. "Roswell is serving as a model city by showing how communities can be a vital part of the solution."

Over 25 national partners and supporting organizations have joined this national effort. And, ***We Can!*** continues to grow by adding new partners, program materials, community sites, cities, and counties with the hope that, together, ***We Can!*** prevent childhood obesity.

More information about the ***We Can!*** program can be found at *http://wecan.nhlbi.nih.gov* or by calling 1-866-35-WeCan.

This road sign is posted in We Can! Cities around the country, such as Roswell, Georgia. The sign designates communities that are participating in efforts to help their children maintain a healthy weight.



Transmission electron micrograph showing *Escherichia coli (E. coli)* bacteria attaching to intestinal epithelial cells (arrows). This strain of *E. coli* is a major cause of infantile diarrhea worldwide, which is associated with a high rate of mortality. Recent NIDDK-sponsored research advances described in this chapter shed light on how these and other bacterial strains interact with intestinal cells, contributing either to human health or disease.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract exact a significant toll on many Americans each year. For example, approximately 135 million people each year suffer from non-food-borne gastroenteritis, a typically infectious inflammation of the GI tract associated with such symptoms as diarrhea, nausea, and vomiting.[1] Additionally, liver and biliary diseases affect a large portion of the population and represent a huge burden, in terms of quality of life as well as health care costs, such as the estimated 6 billion dollars spent annually in the U.S. on gallbladder disease care.[1] NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.

Diseases of the GI tract include inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis. These diseases are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. IBD often strikes early in life, with a peak age of onset in adolescence or young adulthood. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued discovery of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences. Research on controlling intestinal inflammation has potential benefits not only for patients with inflammatory bowel diseases, but also for those at risk of developing colorectal cancer.

Intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroparesis is another functional bowel disorder that is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their healthcare providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued

[1] *Sandler RS, et al: Gastroenterology 122: 1500-1511, 2002.*

advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the gastrointestinal tract are increasingly appreciated as powerful players in maintaining or tilting the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with host cells, and with nutrients ingested by their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract.

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

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

GENETICS OF INFLAMMATORY BOWEL DISEASES

Autophagy Is Implicated in Crohn's Disease:

Scientists have recently identified a gene that increases susceptibility for Crohn's disease (CD), a major form of inflammatory bowel disease (IBD). The gene is involved in autophagy, a process cells use to eliminate unwanted cellular components by capturing and degrading them into molecules that can be recycled by the cell. This exciting discovery was made in a two-phase study supported by the NIDDK IBD Genetics Consortium.

As described in the Cross-Cutting Science chapter, genome-wide association scans are now possible which can screen individuals' complete genomes for hundreds-of-thousands of small mutations, or gene variants. In the first phase of the study, members of the IBD Genetics Consortium conducted a screen using this state-of-the-art technology to identify genetic variants that contribute to CD. The scans were performed using the DNA of 547 CD patients and 548 healthy volunteers. Surprisingly, when the genetic variants of the two groups were compared, a relatively rare variant of the IL-23 receptor gene was identified that protects against CD. (See highlights from Dr. Judy Cho's Scientific Presentation, which appears later in this chapter.)

CD is a complex genetic disease caused by inappropriate immune responses to bacteria that naturally reside in the intestine. Because CD is a complex disease involving several genetic and environmental factors, the scientists conducting this study believed that some of the genes contributing to CD may have only a modest effect on disease risk and that larger study cohorts would be required if these subtle gene variants were to be detected. Therefore, a second phase of the study was conducted, which increased the sizes of the study cohorts to a total of 946 CD patients and 977 healthy volunteers. Analyses of these larger scans identified the new CD autophagy gene. Several other significant CD-associated genetic variants were identified in these scans as well. The discovery of an autophagy gene associated with CD is an important step in understanding this complex disease. Additionally, autophagy and the signaling pathways used to initiate this process can now be explored as targets for novel drugs designed to prevent and treat CD.

Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, and Brant SR: Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 39: 596-604, 2007.

UNDERSTANDING INTERACTIONS BETWEEN BACTERIA AND THE GASTROINTESTINAL TRACT

Of Mice, Fish, and Men—Multi-Species Studies Explore Impact of Intestinal Microbes:

Three recent studies using animal models and human samples yield new insights and renewed curiosity about the impact of the trillions of microbes that inhabit the human intestine. These studies combine new genetic knowledge and techniques with the most useful animal and bacterial models available to shine a light on the interior world of intestinal microbes.

One study characterized the functions and evolutionary adaptations of a type of bacterium that is abundant in the human intestine and known to influence host nutrient digestion. The bacterium is called *Methanobrevibacter smithii,* or *M. smithii*. This bacterium was shown in previous experiments to increase the efficiency of host digestion. The result was more calories absorbed and a heightened risk for obesity. (Additional information on how gut microbes influence obesity development is presented in the chapter on "Obesity.") Building upon these findings, researchers sequenced the bacterium's genome and compared it to other bacterial strains to identify genes that are specific to *M. smithii* and point to its unique functions inside the human intestine. They then used a mouse model that had been raised in a sterile environment to keep its intestine completely free of bacteria. In this model, they identified the unique RNAs expressed and metabolic functions performed when *M. smithii* was introduced into the mouse gut. The researchers concluded this set of investigations by exploring some potential targets in the *M. smithii* genome for future drug discovery, specifically inhibitors of the bacterial genes that enhance their host's ability to harvest energy from the diet.

In another study, resourceful researchers used advanced genomic tools and data, some swimming bacteria, and a see-through fish to track the movements and host impacts of intestinal bacteria. Their findings contrasted with previous research, which presented a static view of bacterial behavior in the gut. When watching "live" through a microscope, researchers could see the bacteria traveling through the intestine of the naturally transparent zebrafish, *Danio rerio*. Similarly to the mouse model in the *M. smithii* study, the zebrafish were raised in a sterile environment until the introduction of the intestinal bacteria *Pseudomonas aeruginosa*. This type of bacteria is usually thought of as harmful, in part because of its presence in patients with inflammatory bowel disease and cystic fibrosis. However, recent research has challenged this belief by showing beneficial effects in fish. For these experiments, the bacteria were made to glow by introducing a fluorescent gene for easier visualization. Using this simplified, highly visible system, scientists were able to observe how the bacteria navigated the intestine, swimming with their flagellum—a whip-like tail. To test the impact of this bacterial colonization on the fish, some of the bacteria were hobbled by disabling their flagellum. Immobilization of the bacteria correlated with a drop in immune responses, demonstrating the bacteria's beneficial effects on fish immunity.

A third study combined both the mouse and zebrafish models in a reciprocal transplant experiment to answer fundamental questions about the evolutionary origins and inter-species differences of intestinal microbial communities. Mammals and fish typically host very different types of microbes in their intestines. By swapping intestinal contents between these species, scientists hoped to determine how the host habitat shapes the microbial community. In these experiments, both animals were raised either under sterile conditions to keep their intestines free of microbes, or in a normal environment where microbes naturally colonize the intestine. Then, intestinal contents from a normally raised zebrafish were transplanted into a sterile mouse, and vice versa (sterile zebrafish were colonized with mouse intestinal contents). Days after this initial colonization, the intestinal contents were sampled from the transplanted animals, and DNA was sequenced to identify the bacterial species present. Remarkably, the researchers found that each animal shaped the foreign microbial community to more closely resemble its

native mix of intestinal microbes. For example, in the transplanted mouse intestine, bacteria that are abundant in the native mouse intestinal community, such as the Firmicutes, were amplified, while the zebrafish's dominant bacteria, known as Proteobacteria, were diminished. A similar phenomenon was observed in the transplanted zebrafish intestine, where the Proteobacteria, a minor type of species in the mouse's intestine, was substantially amplified in the zebrafish, while other species were reduced or absent.

Each of these studies reveals a fascinating facet of intestinal microbes, from their origins to their impact on the host's everyday functions, such as absorbing nutrients and maintaining a healthy immune system. Future studies will continue these explorations of bacterial-host interactions in the intestine and their relationship to human health.

Rawls JF, Mahowald MA, Ley RE, and Gordon JI: Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell 127: 423-433, 2006.

Rawls JF, Mahowald MA, Goodman AL, Trent CM, and Gordon JI: In vivo imaging and genetic analysis link bacterial motility and symbiosis in the zebrafish gut. Proc Natl Acad Sci USA 104: 7622-7627, 2007.

Samuel BS, Hansen EE, Manchester JK, Coutinho PM, Henrissat B, Fulton R, Latreille P, Kim K, Wilson RK, and Gordon JI: Genomic and metabolic adaptations of Methanobrevibacter smithii to the human gut. Proc Natl Acad Sci USA 104: 10643-10648, 2007.

Mechanism of Enteropathogenic *E. coli* Infection:

NIDDK-supported scientists recently uncovered, in greater detail than ever before, the strategies used by a particular food-borne bacteria to cause intestinal disease. The bacteria, known as enteropathogenic *E. coli* (EPEC), cause severe watery diarrhea, particularly in infants living in developing countries. In fact, EPEC causes far more illness and death worldwide than the *E. coli* strain O157:H7, which was responsible for the high-profile, food-related illnesses in the United States in recent years. Unlike other types of *E. coli*, EPEC does not cause illness by releasing a toxin or entering host cells, but rather by attaching to the host's intestinal cells and directly affecting their function. However, until recently, it was not fully understood how infection with EPEC caused this occasionally deadly disease.

Diarrhea often occurs when sodium chloride (NaCl) cannot be absorbed from nutrients passing through the intestinal tract. NaCl absorption is accomplished by its transport across intestinal cell membranes in a process known as coupled ion exchange. This process maintains a neutral charge in intestinal cells while absorbing the charged sodium (Na+) and chloride (Cl-) ions. In the current study, scientists gained new insight into EPEC-induced diarrhea by studying the chloride exchange process in mouse models and in cultured human intestinal cells infected with EPEC using radioactively labeled chloride. From these studies, they determined that chloride uptake by intestinal cells is diminished in the presence of EPEC. Further investigation demonstrated that EPEC secrete bacterial molecules into host cells that disrupt the transport of a chloride ion exchanger protein, DAR, to its proper location in the cell's membrane thus preventing the uptake of chloride. The resulting decrease in cellular chloride creates an imbalance of sodium and chloride ions within the cells, which in turn precipitates watery diarrhea. Based on these studies, scientists designed a molecular model detailing the cascade of events that occur during EPEC infection. This valuable model of EPEC's *modus operandi* and its consequences will enable the development of new drugs to treat and to prevent infant mortality caused by EPEC infection.

Gill RK, Borthakur A, Hodges K, Turner JR, Clayburgh DR, Saksena S, Zaheer A, Ramaswamy K, Hecht G, and Dudeja PK: Mechanism underlying inhibition of intestinal apical Cl/OH exchange following infection with enteropathogenic E. coli. J Clin Invest 117: 428-437, 2007.

LIVER DISEASE RESEARCH

Comparing Outcomes of Liver Transplantation Procedures in Patients with Hepatitis C:

Researchers who are following a large group of liver transplant recipients are gaining insights into the best use of these procedures in patients with hepatitis C. Liver transplantation is often the only treatment option available for patients with advanced liver disease due to hepatitis C, but donor livers are in limited supply. Use of portions of the liver from living donors has

expanded the pool of organs available, but may present additional complications not experienced when using organs from deceased donors. These complications could make the procedure riskier for patients with advanced liver disease, such as those individuals with chronic hepatitis C. Ongoing follow-up of patients at nine U.S. liver transplant centers in the NIDDK-sponsored Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) recently provided important information to guide the use of this procedure in patients with hepatitis C. By tracking outcomes for about 3 years after the transplant, the researchers uncovered key factors that contributed to success of the transplants in patients receiving organs from living or deceased donors. Chief among these factors was the level of experience of the transplant center in performing the more novel living donor procedure. Once a transplant center was sufficiently experienced in performing living donor liver transplants, the procedure outcome was as successful as one using a deceased donor organ in patients with hepatitis C. Based on this research, patients with hepatitis C needing a liver transplant should have equally successful outcomes with organs from either a living donor or a deceased donor, provided the transplant center staff are proficient at both procedures. This flexibility greatly increases the chances that these patients will receive a potentially life-saving transplant.

Terrault NA, Shiffman ML, Lok ASF, Saab S, Tong L, Brown RS Jr, Everson GT, Reddy KR, Fair JH, Kulik LM, Pruett TL, Seeff LB, and the A2ALL Study Group: Outcomes in Hepatitis C virus-infected recipients of living donor vs. deceased donor liver transplantation. Liver Transpl 13: 122-129, 2007.

INHERITED DISEASES

Copper Availability and Menkes Disease:

Researchers have delineated a role for copper in nerve cell activation and early central nervous system development. Copper is an essential nutrient in the diet that is rapidly absorbed by the stomach and intestine into the circulation and transported to the liver. As a critical component of many enzymes, it plays a key role in cellular respiration, iron oxidation, antioxidant defense, connective tissue formation, and the formation of nerve-conduction factors. The liver is the primary site of copper storage, and normal body levels can be reduced if copper transport is impaired, or if clinical conditions are present that cause malabsorption. The maintenance of appropriate levels of copper is critical for brain function, as well as for the development and activity of the nervous system. Clinical and experimental studies show that copper deficiency during pregnancy leads to neurologic problems and abnormal organ development in offspring.

Mutations in the gene encoding a copper-transporting enzyme known as atp7a result in Menkes disease, an X-linked, neurodegenerative disorder that can cause seizures, failure-to-thrive, and, ultimately, death. Normally, atp7a helps to maintain sufficient copper levels inside cells, such that the presence of a mutated *atp7a* gene limits copper availability. Previous clinical studies of patients with Menkes disease have shown points of contained degeneration of the gray matter of the cerebral cortex, and neuronal loss most pronounced in two specific areas of the brain—the hippocampus and cerebellum.

To uncover the specific mechanism by which copper availability affects neuronal activation, researchers over-stimulated the N-methyl-D-aspartate (NMDA) receptor on neurons in the presence or absence of a compound that binds available copper. The overstimulation of neurons—also known as excitotoxicity—is a pathological process by which the cells are damaged and killed. The experiments showed that a lack of available copper causes increased NMDA receptor-mediated excitotoxic cell death, suggesting that copper specifically protects against this type of neuronal death. Furthermore, neurons obtained from a mouse model of Menkes disease, in which the copper-transporting enzyme is synthesized but not functional, revealed an increased sensitivity to excitotoxicity. The increased sensitivity to excitotoxicity was prevented by addition of copper. Thus, these results suggest a functional interaction between NMDA receptor activity and copper availability that could form the basis of a potential treatment for Menkes disease.

To further elucidate copper-related mechanisms for defects in Menkes disease, researchers performed a genetic screen in a zebrafish model in order to investigate the role of copper in notochord formation, which is similar to early spinal development in humans. Using a genetic screen for embryos exhibiting traits

of copper deficiency, the scientists also discovered a mutation in the zebrafish gene *atp7a*. This zebrafish model of Menkes disease should allow characterization of the development of abnormalities associated with this disorder, and allow high-throughput screens for clinically relevant reagents that restore copper-dependent enzyme activity in spite of *atp7a* mutations. Furthermore, excitotoxic neuronal death from low oxygen levels or blood flow causes significant neonatal morbidity and mortality, and these studies offer possible therapeutic approaches to this problem through manipulation of copper availability. Taken together, these studies underscore the importance of copper availability in the body for ensuring healthy organ development and preventing life-threatening disease.

Schlief ML, West T, Craig AM, Holtzman DM, and Gitlin JD: Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. Proc Natl Acad Sci USA 103: 14919-14924, 2006.

Mendelsohn BA, Yin C, Johnson SL, Wilm TP, Solnica-Krezel L, and Gitlin JD: Atp7a determines a hierarchy of copper metabolism essential for notochord development. Cell Metab 4: 155-162, 2006.

Molecular Link to Autoimmunity in Wiskott-Aldrich Syndrome: Recent findings enhance understanding of the roles of a key protein and a specific population of immune cells in causing the autoimmune disease associated with Wiskott-Aldrich Syndrome (WAS). This syndrome is an inherited disorder of the immune system that is characterized by recurrent infections, bleeding due to low platelet numbers, and eczema. Many patients with WAS also have one or more immune system-related conditions, such as intestinal inflammation, arthritis, or vasculitis. However, a paradox exists in which WAS patients display both immune deficiency (inability to fight off infection) and autoimmunity (inappropriate up-regulated reactivity of immune system to "self"). WAS is caused by a mutation in the gene coding for the WAS protein. This mutated protein impairs the immune cells' ability to transmit signals from receptors on the cell surface to the cytoskeleton. More than 200 unique mutations have been characterized in the *WAS* gene. Patients with the more severe manifestations have mutations in the *WAS* gene that result in loss of protein production. Until recently, most studies have focused on understanding the defects in activation of T cells (a specific type of immune system cell) caused by impairment of WAS protein. Now NIDDK-supported scientists have identified a specific population of T cells that are negatively affected in the absence of WAS protein. This population of T cells—called regulatory T cells—functions to prevent other immune cells from attacking the body's own tissues. The scientists undertook investigations in both humans and mice. Their studies of a WAS patient whose *WAS* mutation spontaneously reverted to a functional form offer new insight into this protein's effect on the stability of regulatory T cells. Previously, this patient had a mutation in the *WAS* gene that resulted in the absence of WAS protein production with a clinical picture of lifelong, recurrent episodes of autoimmune hemolytic anemia. The revertant is predicted to restore the normal protein amino acid sequence and normal WAS protein expression. Indeed, after the mutation reverted to a functional form, the patient exhibited an improved clinical picture with a striking increase in regulatory T cells expressing WAS protein. WAS protein-deficient mice were found to develop high levels of self-reactive molecules (autoantibodies) and autoimmune disease. To gain further insight into WAS, the researchers studied mice in which the *WAS* gene had been deleted. While WAS protein-deficient mice produced normal levels of regulatory T cells in the thymus, there was a preferential loss of regulatory T cells within the overall peripheral T cell pool. These findings indicate that regulatory T cells deficient in WAS protein do not proliferate or survive as well as normal cells with a functional WAS protein. These studies provide important information regarding how the WAS protein sustains regulatory T cells, and how alterations in these key factors contribute to the autoimmune disease experienced by patients with WAS. This research further contributes to our overall understanding of the role of the regulatory T cell in preventing autoimmune disease.

Humblet-Baron S, Sather B, Anover S, Becker-Herman S, Kasprowicz DJ, Khim S, Nguyen T, Hudkins-Loya K, Alpers CE, Ziegler SF, Ochs H, Torgerson T, Campbell DJ, and Rawlings DJ: Wiskott-Aldrich syndrome protein is required for regulatory T cell homeostasis. J Clin Invest 117: 407-418, 2007.

BARRETT'S ESOPHAGUS RISK FACTORS

Elucidating Risk Factors for Barrett's Esophagus:

Scientists report that extra abdominal fat may be a risk factor for Barrett's esophagus. Barrett's esophagus is a precancerous condition where the cells lining the esophagus change in shape and organization. Short of surgical removal of the esophagus, there is no effective cure for the condition. Known risk factors for Barrett's esophagus include being male, Caucasian, or over the age of 40. Individuals with gastroesophageal reflux disease (GERD), in which stomach acid flows backward into the esophagus, also are at increased risk of developing Barrett's esophagus. In turn, the condition puts patients at greater risk of developing esophageal adenocarcinoma, which is rapidly increasing in the United States. Therefore, research to further understanding of the risk factors for Barrett's esophagus may yield information that also would provide insight into the development of esophageal adenocarcinoma.

Scientists recently examined the association between obesity and abdominal girth and the occurrence of Barrett's esophagus and GERD. Obesity is thought to be a risk factor for GERD, and extra abdominal fat could directly promote acid reflux by placing added pressure on the stomach. Obesity was determined using body mass index (BMI), a measure that takes both height and weight into account, and abdominal girth was measured simply as waist size. In this study, while there was no association of BMI with Barrett's esophagus, larger abdominal circumference was moderately associated with the condition. The association was strongest in individuals with no GERD symptoms. When GERD symptoms were included in the analysis, the association between abdominal girth and Barrett's esophagus was decreased, which was expected as GERD symptoms may partly mediate the effect of abdominal girth in the development of Barrett's esophagus. These data suggest that larger abdominal girth may be a risk factor for both GERD and Barrett's esophagus. Although the mechanism by which extra abdominal fat raises the risk of these conditions is unknown, it could act by increasing pressure on the stomach and contributing to GERD symptoms through effects on gastrointestinal mobility. The results of this research suggest that larger abdominal circumference, but not overall obesity, is a risk factor for Barrett's esophagus. Based on these findings, reduction of waist size may be advisable for patients at high risk of developing this condition or subsequent esophageal carcinoma.

Corley DA, Kubo A, Levin TR, Block G, Habel L, Zhao W, Leighton P, Quesenberry C, Rumore GJ, and Buffler PA: Abdominal obesity and body mass index as risk factors for Barrett's esophagus. Gastroenterology *133: 34-41, 2007.*

National Commission on Digestive Diseases

Diseases of the digestive system span a wide range of conditions—from functional gastrointestinal and motility disorders, inflammatory bowel disease, and celiac disease, to liver and gallbladder diseases, to pancreatic diseases and GI cancers. Collectively, these diseases represent an enormous public health burden. A strong commitment to advancing research is required to combat digestive diseases.

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

As part of its charge, the Commission is assessing the state-of-the-science in digestive diseases and the related NIH research portfolio, in order to identify research challenges and opportunities for inclusion in its long-range research plan for digestive diseases. The Commission's efforts benefit from the diverse expertise of its members, who represent the academic and medical research and practice communities, the patient advocacy community, and the NIH and other Federal health agencies. In 2006, the Commission initiated a research planning process through two public meetings held near the NIH and by engaging in activities such as: (1) defining the topic areas within digestive diseases research that comprise the research plan, (2) assigning Commission members to chair topical Working Groups, (3) conducting an open call for additional experts to serve as Working Group members, and (4) laying the foundation for the Working Groups' deliberations by teleconference to identify research goals and other recommendations. Dr. Zerhouni addressed the Commission members at their November 2006 meeting to express his support and appreciation for their efforts.

During the Commission's public meeting in June 2007, Working Group chairs presented their Groups' research recommendations for specific digestive diseases, along with steps to achieve the proposed goals. The chairs then provided the Commission members with content for the topic-specific chapters of the research plan. At the following meeting in November 2007, in Chicago, Illinois, the Commission considered the entire draft research plan and invited public comments. A formal public comment period held after the November meeting invited additional stakeholder input on the draft research plan, which was posted on the internet. Following incorporation of public input and preparation of the final publication, release of the completed research plan is anticipated in 2008. The Commission's 10-year research plan will guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases.

Additional information about the Commission can be found on its website: *http://NCDD.niddk.nih.gov*

Functional Dyspepsia Treatment Trial Launched

The NIDDK is sponsoring a new clinical trial to explore the causes and most effective treatment of a common digestive condition known as functional dyspepsia. Functional dyspepsia is a type of indigestion for which symptoms can include severe stomach pain or great discomfort after eating. These symptoms are thought to result from abnormal muscular activity in the stomach, which may in turn result from abnormal neural activity within the stomach or between the brain and the gut. Current treatments are limited to food restriction or drugs that affect muscular activity in the stomach.

Now a new clinical trial has been launched to test potential therapeutic agents for functional dyspepsia that act on the brain and gut. The Functional Dyspepsia Treatment Trial (FDTT) will test two FDA-approved drugs—amitriptyline and escitalopram. These drugs are traditionally prescribed as anti-depressants, but the trial will determine if they can also relieve stomach pain or discomfort after meals in patients with functional dyspepsia. Additional goals of the trial include the discovery of particular genes that predict response to these treatments and a determination of whether the response is "durable" over the long term, even after patients stop taking the drugs for 6 months. Recruitment of patients for the trial began in January 2007 and will continue for 5 years. Patients participating in the trial are between the ages of 18 and 75 and have not experienced relief from their functional dyspepsia symptoms with previous treatments.

More information about functional dyspepsia and other common forms of indigestion is available through the National Digestive Diseases Information Clearinghouse at: *http://digestive.niddk.nih.gov*

STORY OF DISCOVERY

Advances in Inflammatory Bowel Diseases Research

NIDDK support for research on genetics and immunology of the inflammatory bowel diseases (IBD) is paving the way to the development of unique and effective therapies for patients who suffer from these diseases.

IBD was described in the medical literature as early as the mid-18th Century, but it was not until the mid-20th Century that the two major subtypes of IBD—Crohn's disease (CD) and ulcerative colitis (UC)—were identified and distinguished by the area of the intestine they affect. The incidence of these diseases in western, industrialized societies increased dramatically during the 20th century. These are painful and debilitating diseases, characterized by chronic, intermittent intestinal inflammation. In CD, inflammation can occur anywhere in the alimentary tract and sometimes in other sites, but most often occurs in the end of the small bowel and beginning of the large bowel (colon). In UC, the site of inflammation is restricted to the colon, or large intestine. The leading theory for the cause of IBD is that inflammation is triggered by inappropriate immune responses to bacteria that naturally reside in the intestine and that the underlying predisposition to these inappropriate immune responses is caused by multiple interacting genes. Under normal circumstances, most bacteria residing in the gut have a beneficial or benign effect on their host, but an overly active immune system may be provoked by these bacteria in IBD.

Genetic Factors in IBD Uncovered

Studies of human twins and of animals have confirmed that genetic factors contribute to IBD. Some gene variants are specifically associated with either CD or UC, while others are involved in both diseases. The importance of genetic factors is also reflected in family studies showing the incidence of IBD to be higher among family members.

A major research breakthrough on the genetics of IBD came in 2001, with the discovery of the first IBD-associated gene, called *NOD2*. The *NOD2* gene was found to be associated solely with CD, not with UC. This landmark research, which was supported by the NIDDK, represents one of the earliest, most well-established associations in complex genetic disorders. The product of *NOD2* is a cellular protein found in immune cells, called monocytes, and in cells lining the intestinal wall. Although the mechanisms underlying the relationship of the *NOD2* gene variant to CD are not yet fully understood, the NOD2 protein is known to activate communication (signaling) pathways in response to components of bacterial cell walls, leading to a variety of immune responses.

Building on this important finding, the NIDDK in 2002 established the Inflammatory Bowel Disease Genetics Consortium (IBDGC). (For more information on the Consortium, see highlights from a Scientific Presentation by Consortium investigator Dr. Judy Cho, which appears later in this chapter).

The Consortium's efforts were greatly enabled by resources provided by the NIH-sponsored Human Genome Project and the International HapMap Project, which were major drivers in propelling research on human genetics. The Human Genome Project sequenced the 3 billion nucleotide base pairs of the human genome, a monumental effort that concluded in April 2003. Data from this project were made available to scientists around the globe to facilitate the pursuit of medical research. The International HapMap Project, published in 2005, is a catalogue of common small genetic variations called SNPs (single nucleotide polymorphisms) that occur in the nucleotide (or letter) sequences of individuals' DNA. The Genome and HapMap projects have been accompanied by great strides in the development of new rapid biomedical technologies so that hundreds

of thousands of SNPs can now be determined in single DNA samples. The genome-wide association scan based on these advances has become the cutting-edge technology for identifying genes that contribute to human disease, and was used by the Consortium to identify genetic factors in IBD.

Recently, members of the Consortium used this genome-wide association technology in a two-phase study designed to identify additional genes that contribute to CD. In this study, blood samples from CD patients and healthy volunteers were scanned for known genetic variants using over 300,000 SNPs. The first phase of the study was very successful in detecting several significant SNP associations, including a variant of a gene encoding a receptor for the cytokine (an immune system chemical) interleukin-23 (IL-23). Surprisingly, one variant of the gene was shown to protect against CD. Additional studies have shown that the IL-23 receptor is required for CD to develop in animal models.

Because the inflammatory bowel diseases are complex diseases involving the contributions of many genes, it was anticipated that genes also existed that had more subtle associations with IBD, the detection of which would require screening much larger cohorts. Therefore, a second, expanded phase of the study was conducted on a larger population of CD patients and healthy volunteers. In this second phase of the study, scientists discovered another CD associated gene, *ATG16L1*, which is involved in autophagy. Autophagy is a process by which cells capture, degrade, and recycle unwanted cellular material into useful molecules. This process has also been associated with the body's early immune response that is activated by the recognition of bacterial components. The involvement of the autophagy process has been verified by two other scientific research groups. One group identified the autophagy gene, *ATG16L1*, using a different protocol in which 72 SNPs, selected through a screening process, were used in a genome-wide association scan of CD patients and healthy controls. The other group identified a second autophagy gene linked to CD, called *IRGM*, in a major genome-wide association study that scanned 14,000 patients with seven different diseases (2,000 patients for each of the seven diseases) and a shared control set of 3,000 healthy volunteers. The study identified 27 additional disease-related genetic variations, including nine for CD, seven for type 1 diabetes, and three for type 2 diabetes.

Mapping the Molecular Pathways of IBD Development

Discovery of the IBD gene, *NOD2*, provided the first evidence linking this disease to the immune response to bacteria. The NOD2 protein is an intracellular sensor of bacterial wall components. Upon sensing the bacteria, NOD2 activates multiple molecular pathways associated with initial responses by the immune system. Extensive research continues to clarify the roles that pathways stimulated by NOD2 play in the errant activation of immune response associated with IBD.

Research on chemicals utilized by the immune system, including cytokines such as interleukins, has demonstrated the important role of pathways activated by these molecules in IBD development. Identification of the interleukin-23 receptor gene as being associated with the risk of developing IBD coincided with other research investigating the roles of IL-23 and its receptor in autoimmunity, the immune system's inappropriate reaction to the body's own tissues. Studies exploring the causes of inflammation in autoimmune disease have focused on two cytokines, IL-12 and IL-23, which have related structures, but different functions. These two molecules are dimers that each have one identical subunit, as well as one unique subunit. Antibodies against the common, shared subunit of the two cytokines inhibit inflammation in both animals and in human CD. More recent studies in mice have shown that IL-23, not IL-12, is responsible for inflammation.

STORY OF DISCOVERY

In one study examining the roles of IL-12 and IL-23 in IBD, scientists used a mouse model infected with bacteria known to induce inflammation and then analyzed IL-12 and IL-23 subunit expression in the intestine. The mice responded to bacterial infection with increased production of the common subunit of both interleukins and the unique subunit of IL-23, but not the IL-12 unique subunit, demonstrating that inflammation is dependent on IL-23, not IL-12. Furthermore, when antibody was introduced to block the unique IL-23 subunit, inflammation was markedly reduced, confirming that IL-23 is essential for inflammation in the intestine. Confirmation that IL-23, not IL-12, is required for intestinal inflammation was made in another study using two mice strains with double mutations. Both strains contained a mutation that causes them to spontaneously develop inflammation resembling CD. Additionally, the IL-12 unique subunit was inactivated in one strain and the IL-23 unique subunit was inactivated in the second strain. Mice with mutations in the IL-12 unique subunit developed colitis; however the IL-23 unique subunit mutants remained disease free, confirming that active IL-23 is essential for intestinal inflammation. These results point to selective targeting of IL-23 as a potential new therapeutic approach for human IBD.

Recent research has also refined our understanding of the types of immune cells involved in IBD, and how they interact with key molecular pathways. IL-23 has been shown to induce the production of other inflammatory cytokines by immune cells called monocytes and macrophages. IL-23 also activates a recently identified subtype of helper T cells (T_H cells), called T_H17 cells. Until recently, only two major subsets of T_H cells had been identified: T_H1 cells, which secrete molecules that destroy intracellular microbes and are associated with CD; and T_H2 cells, which secrete molecules that destroy extracellular microbes and are associated with UC. The newly-discovered T_H17 cells secrete the inflammatory cytokines TNF-alpha, IL-6, and IL-17, and are thought to be particularly important in causing tissue inflammation in immune diseases, including IBD. Thus, not only has IL-23 been implicated as an important cytokine in IBD, it appears that the cytokine acts through a very specific type of T cell that has only recently been identified. These discoveries suggest important new pathways to be explored to develop treatments for CD.

New Treatments for IBD

The elucidation of new disease genes and the molecular responses they initiate is key to developing drugs that prevent and treat IBD. Two examples from recent years involve molecules known as TNF-alpha and PPAR-gamma. The cytokine TNF-alpha is now recognized as a major factor in the inflammatory immune responses associated with IBD. The drug infliximab was the first recombinant antibody designed to bind to TNF-alpha, thereby preventing it from engaging with receptors that activate inflammatory responses. Infliximab was initially thought to be effective only in treating and maintaining remission of CD, but has now been show to be an effective treatment of UC.

The peroxisome proliferator activated receptor-gamma (PPAR-gamma) regulates gene expression in the nuclei of immune cells and epithelial cells that line the colon and is known for its effects on tumor suppression in the colon and on attenuation of colitis. PPAR-gamma expression was found to be impaired in cells lining the colons of UC patients, indicating a potential role in the treatment UC. Mutant mice with minimal expression of PPAR-gamma in their colon epithelial cells were given a substance that induces colitis, in order to determine if PPAR-gamma plays a protective role against developing UC. Mutant mice exhibited higher levels of molecules that promote inflammation and increased susceptibility to experimental colitis when compared with control mice. Rosiglitazone, a drug used for the treatment of type 2 diabetes, activates the PPAR-gamma receptor. When rosiglitazone was administered to the mice, the severity of the induced colitis was decreased and cytokine production was suppressed

in both mutant and control mice, demonstrating that PPAR-gamma plays a role in protecting against colitis. Because administration of rosiglitazone was effective in reducing colitis symptoms in mutant mice expressing minimal levels of PPAR-gamma, as well as control mice, it is possible that rosiglitazone may also act independently of PPAR-gamma in suppressing inflammation.

The efficacy of rosiglitazone in treating UC in humans was recently tested in a multicenter clinical trial supported by the NIDDK. Patients participating in the trial had mild-to-moderate UC and had been previously treated with the drug 5-aminosalicylate, the most common treatment for UC, but had not responded well or were intolerant to the drug. After receiving either rosiglitazone or a placebo, patients were assessed for improvement in their condition. After 12 weeks, 44 percent of patients given rosiglitazone had clinical remission compared to 23 percent of patients given placebo. These data demonstrate that rosiglitazone is effective in the short-term treatment of patients with mild-to-moderate UC who did not benefit from other treatments. Further long-term studies must still be conducted to assess use of this class of drug as a maintenance therapy for UC, and to determine whether they provide an additional new treatment option for patients suffering from UC.

Genetics of Inflammatory Bowel Diseases (IBD): IL23R as an IBD Susceptibility Gene

Dr. Judy Cho

Dr. Judy Cho is an Associate Professor in the Department of Medicine and Genetics at the Yale School of Medicine and the Director of Yale's Inflammatory Bowel Disease Center. A leader in the field of inflammatory bowel disease (IBD) research, Dr. Cho and her colleagues are widely recognized for their 2001 discovery of the first known gene to increase susceptibility to Crohn's disease—the NOD2/CARD15 gene. Dr. Cho is the Chair of NIDDK's Inflammatory Bowel Disease Genetics Consortium Steering Committee and Principal Investigator of the Consortium's Data Coordinating Center. Recently, the NIDDK IBD Genetics Consortium identified IL-23R as another IBD susceptibility gene. Dr. Cho presented this clinical research study to the NIDDK Advisory Council at their February 2007 meeting. The following are highlights from her presentation. (Additional information on IBD-related research conducted by Dr. Cho and other NIDDK-sponsored researchers is presented in this chapter's "Story of Discovery.")

The inflammatory bowel diseases are chronic, intermittent intestinal inflammations which are thought to result from inappropriate responses by the immune system to bacteria normally found in the intestine. Symptoms include diarrhea, abdominal pain, intestinal bleeding, and, in cases of childhood onset, growth retardation. IBD occurs frequently in young people—with a peak age of onset between 15 and 30 years of age. Because IBD is largely seen in industrialized societies, some researchers have suggested that it may be associated with changes in intestinal microbial populations during the industrialization process.

The two major subtypes of IBD are ulcerative colitis (UC) and Crohn's disease (CD), which are distinguished by the area of the intestines affected. The site of inflammation with UC is restricted to the colon, or large intestine. In CD, inflammation is found in the small intestine and often affects both the small and large intestines. Ileal CD, which targets a part of the small intestine known as the ileum, is the most common form of Crohn's disease.

Although little is understood regarding its etiology, IBD is known to involve complex interactions between multiple genes, as well as the microbial environment of the intestine. Two genetic associations with IBD had been well-established, the *NOD2* gene, identified by Dr. Cho and her colleagues, and a variant in another area of the genome called *IBD5*. The discoveries of these genetic variations provided two pieces of the complex IBD puzzle, but did not fully explain the incidence of IBD. Thus, the search continued for other genes associated with this disease.

Searching for Additional IBD Genes

The prevalence of Crohn's disease is several times higher in the Ashkenazi Jewish population of European ancestry than in the non-Jewish population of European ancestry. In this genome-wide association study, over 300,000 naturally occurring genetic variations were screened in Ashkenazi Jewish and non-Jewish patients and healthy controls. These variations, known as SNPs (single nucleotide polymorphisms) are small differences in an individual's DNA sequence that can have varying disease consequences ranging from causing major genetic diseases such as cystic fibrosis or sickle cell anemia to more subtle effects that alter disease risk. SNPs are valuable disease biomarkers used in both research and clinical diagnoses.

SCIENTIFIC PRESENTATION

The study began with a genome-wide screen of non-Jewish ileal CD patient and control cohorts. Ileal CD patients were selected exclusively to minimize genetic differences within the patient cohort. The screen identified three SNPs having highly significant associations with CD. Two of the three SNPs were located in the previously identified CD susceptibility gene, *NOD2*. However, the third was located in the IL23 receptor gene (*IL23R*). Surprisingly, this uncommon gene variant was found to confer protection against CD.

Ileal CD patients of Ashkenazi Jewish ancestry and their controls were then screened for *IL23R* markers. Highly significant differences were observed in the frequency of the protective marker in the two study groups. For example, this marker was identified in only two percent of CD patients in contrast to seven percent of the controls, supporting its protective role in preventing CD.

Following the two screens, the research team conducted a study to determine the frequency of the transmission of *IL23R* markers in nuclear IBD families consisting of children who were affected by IBD (CD, UC, and indeterminate IBD) and both of their parents. The study revealed that the protective variant of *IL23R* was much less likely to be passed down from parents to their IBD-affected children. Both Jewish and non-Jewish families with this marker were protected against developing CD; however, only the non-Jewish population showed a similar protective effect against UC.

The unexpected identification of a gene variant that protects against the risk of IBD has given new insights into the molecular underpinnings of this disease. These findings substantiate a hypothesis, supported by recent immunological studies, that the *IL23R* gene is required for the manifestation of clinical IBD. Importantly, these research results also provide potential therapeutic targets for its prevention and treatment.

The Inflammatory Bowel Disease Genetics Consortium (IBDGC)

In 2002, the NIDDK established the Consortium to provide the research resources necessary to take advantage of the wealth of genetic information provided by the NIH-sponsored Human Genome Project in elucidating the disease mechanisms of IBD. The infrastructure established to accomplish the Consortium's mission includes a Data Coordinating Center, which oversees genetic analysis, database analysis, and coordination between six Genetic Research Centers. The Centers recruit patients and healthy volunteers for IBD study cohorts, submit patient blood samples and phenotype data to a repository, and conduct genetic research studies. Governance of the Consortium is provided by a Steering Committee consisting of Consortium scientists and a NIDDK health science administrator.

As Chair of the Steering Committee and head of the Data Coordinating Center and of one of the research centers, Dr. Cho has played a significant role in the development of the Consortium. In describing the Consortium's major advantages, Dr. Cho identified:

- Synergy of expertise provided by gastroenterologists who are primarily interested in IBD and geneticists whose interests include IBD and complex disorders;
- Stringent quality control attained through sample and data uniformity;
- Ability to recruit the large numbers of patients required to identify the genes responsible for IBD;
- Availability of resources necessary for high risk, high priority projects—including genetic studies of disease differences in minority populations; and
- Knowledge of priorities and opportunities provided by NIH oversight.

The Consortium has developed collaborations with outside investigators, providing valuable data, genotyping services, and research resources. For

example, immortalized cell lines are being derived from patients' blood samples. These cell lines and the DNA extracted from them will be linked to the patients' phenotypic data and stored for use in Consortium research projects. They will also be made available to the broader scientific community. Additionally, control datasets representing different populations are being analyzed and made available on the web. This approach provides major research effectiveness and cost-saving advantages for future IBD studies.

A Vision for Future IBD Research

Dr. Cho described her vision for the future of IBD research and her major priorities. These include developing models of disease risk, developing biomarkers, predicting disease course, and finding ways to prevent disease. These endeavors all have as their underpinnings the identification of the multiple genes that contribute to IBD and the elucidation of their interactions with each other and their environment. Even genes with limited direct associations with IBD may have significant biological consequences that must be considered in designing important risk models. Biomarkers that reflect genetic variation and the molecular consequences of gene expression are important research indicators of disease risk, disease prognosis, and patient response to therapies. Thus, biomarkers will serve as major drivers in the development of new approaches to the prevention and cure of IBD.

The IBD genome-wide association study presented by Dr. Cho has continued to yield important genetic discoveries. An expansion of this study identified three new IBD susceptibility genes which are described in the IBD Story of Discovery, also in this chapter.

Howard Klein

Taking Life—and Hepatitis B—By the Horns

Two degrees from the Juilliard School of Music…. Classical pianist….Former music critic for *The New York Times*….Director of the Rockefeller Foundation's arts program….Board president for the Carter Family Memorial Music Center Foundation in Virginia….Linguist….Wine connoisseur and collector….Happily married and father of two sons and one stepson….

It's fair to say that 76-year-old Howard Klein is an accomplished Renaissance man. He's also a person with hepatitis B who credits the quality of life he currently enjoys to the treatment and personal attention he has received from clinical trials sponsored by the NIDDK.

Howard Klein

About Hepatitis B

The virus that causes hepatitis B is one of at least five different hepatitis viruses (hepatitis A to E virus) that can result in liver disease in humans. Hepatitis B makes the liver inflamed and swollen. Over time, the virus compromises the liver's ability to perform its bodily functions, which include the organ's ability to fight infections, stop bleeding, process drugs and toxins in the blood, as well as store energy for when the body needs it. In its most extreme forms, hepatitis B can lead to severe liver damage, liver cancer, and the need for a liver transplant.

Thanks to research supported by the NIH and other organizations, hepatitis B has been effectively prevented and controlled in the United States and many other countries through vaccines that protect against the virus and public health education programs. Screening of blood donations also has contributed to a reduction of hepatitis B cases. However, the disease is far from entirely eradicated, especially in individuals who live in or emigrate from countries where the disease is more common, such as parts of Asia.

It's fair to say that 76-year-old Howard Klein is an accomplished Renaissance man. He's also a person with hepatitis B who credits the quality of life he currently enjoys to the treatment and personal attention he has received from clinical trials sponsored by the NIDDK.

Because it is a blood-borne disease, hepatitis B can be transmitted in several different ways (see sidebar). Many people who are infected don't know it because they feel perfectly healthy. Howard, for example, never manifested any symptoms of hepatitis B prior to diagnosis. It was diagnosed through follow-up blood work for another serious illness he was experiencing. That's where Howard's story begins.

Howard's Story

In 1984, Howard was diagnosed with a severe case of lymphoma, a cancer of the lymphatic system. At the time, he was told that he had a 20 percent chance of surviving the disease. His weight

PATIENT PROFILE

dropped to 125 pounds and he required a massive blood transfusion to save his life. Howard believes he contracted hepatitis B through the transfusion, and his doctors agree. However, because he manifested no symptoms, he wasn't diagnosed with hepatitis B until 1995, when his lymphoma was in remission.

Thanks to research supported by the NIH and other organizations, hepatitis B has been effectively prevented and controlled in the United States and many other countries through vaccines that protect against the virus and public health education programs. However, the disease is far from entirely eradicated…

"I was in California on a wine-tasting trip with one of my sons when I got a call from my doctor," Howard says in a somewhat amused tone of voice. "He'd just received the results of my blood work and said I had serious liver problems and not to drink any wine." His doctor also recommended that Howard go to the NIDDK, one of 27 research institutes of the NIH. Howard was finally diagnosed with chronic hepatitis B. Since 1996, Howard has taken part in several NIDDK clinical trials that have tested various pharmaceutical agents for treatment of hepatitis B.

At NIDDK, Howard started out on a drug called lamivudine, which is taken orally once a day. The then experimental treatment looked promising, but, over time, Howard became resistant to the drug. Next, he was put on an interferon drug, a synthetic version of the naturally produced protein used by the body to fight viruses by boosting the immune system. Interferon attempts to prevent the hepatitis B virus from further damaging the liver by inactivating the virus and removing it from the body. This drug is administered much like insulin is for diabetes, with self-injections either into the stomach or leg, which Howard found discomforting.

"I really had problems with self-injecting interferon into my body," he says. As it turned out, his discomfort turned out to be a moot point. His interferon treatment followed the same path as the lamivudine. At the beginning of treatment, Howard responded well, but ultimately, his liver did not respond to the interferon and the hepatitis B virus remained active.

In the meantime, prior to and during the early years of participating in the NIDDK clinical research, Howard's liver, as a result of his chronic hepatitis B, was undergoing an unusually rapid onset of cirrhosis. Cirrhosis occurs when scar tissue replaces normal, healthy liver tissue, blocking the flow of blood through the organ and preventing it from working as it should. Cirrhosis is a leading cause of death in the United States.

"In 2000, my doctor at NIH showed me how deadly my cirrhosis had become. I was really bad off," says Howard. Howard's hepatitis was having an adverse impact on his life and career as a professional musician. "The years 2000 to 2003 were particularly difficult," adds Howard. "Music is very demanding because your brain has to think ahead. You're not just in the moment; you know what's coming and you have to be there. When I'd try to learn a new piece of music or perform, I always felt fatigued and sluggish. No pain, just a general sense of malaise." In 2003, however, Howard was put on a combination drug therapy of lamivudine and adefovir dipivoxil, which, like lamivudine, is taken orally—and it worked.

Howard has benefited from the rapid expansion in recent years of pharmaceutical agents available to treat hepatitis B. The variety of hepatitis B drugs means that, if patients eventually develop resistance to long-term treatment with one drug, they can be switched to another one or put on a combination of drugs that is effective.

"God, I'm lucky!"

"As a result of the combination drug therapy, Howard's prognosis is excellent," says Theo Heller, M.D., the

physician-scientist who has been following Howard all these years at the NIDDK while Howard participated in research studies. "If we performed a biopsy today, I doubt we'd find any cirrhosis," he adds.

But that doesn't mean that Howard can let down his guard. Although his blood tests are all normal and hepatitis B virus levels are below detection, he still has the hepatitis B antigen in his blood. "If he stops his combination drug treatment, his hepatitis will come straight back," says Dr. Heller.

Howard says he sees 76 as the new 56 and adds that there are still plenty of things he'd like to accomplish in his life. "If you're interested in life, you challenge yourself," he says emphatically.

In the face of all this, Howard remains as productive as ever. In addition to his lead role with the Carter Family Memorial Music Center Foundation, Howard continues to perform as a musician. Last year, for example, he played two concerts at the Goethe Institut in New York with his son, who is an opera singer, performing Schubert's famous song cycle *Winterreise*.

Howard says he sees 76 as the new 56 and adds that there are still plenty of things he'd like to accomplish in his life. "If you're interested in life, you challenge yourself," he says emphatically.

So far, Howard has managed to avoid the most serious repercussions of hepatitis B, including liver cancer or the need for an organ transplant. He is currently in an NIDDK-sponsored study of the natural history of liver disease, which is tracking the long-term effects of treatment, and he credits the NIDDK, through its research programs, for giving him this "extended" life opportunity.

"God, I'm lucky!" Howard says in a voice full of energy, hope, and promise.

Hepatitis B spreads by contact with an infected person's blood, semen, or other body fluids.

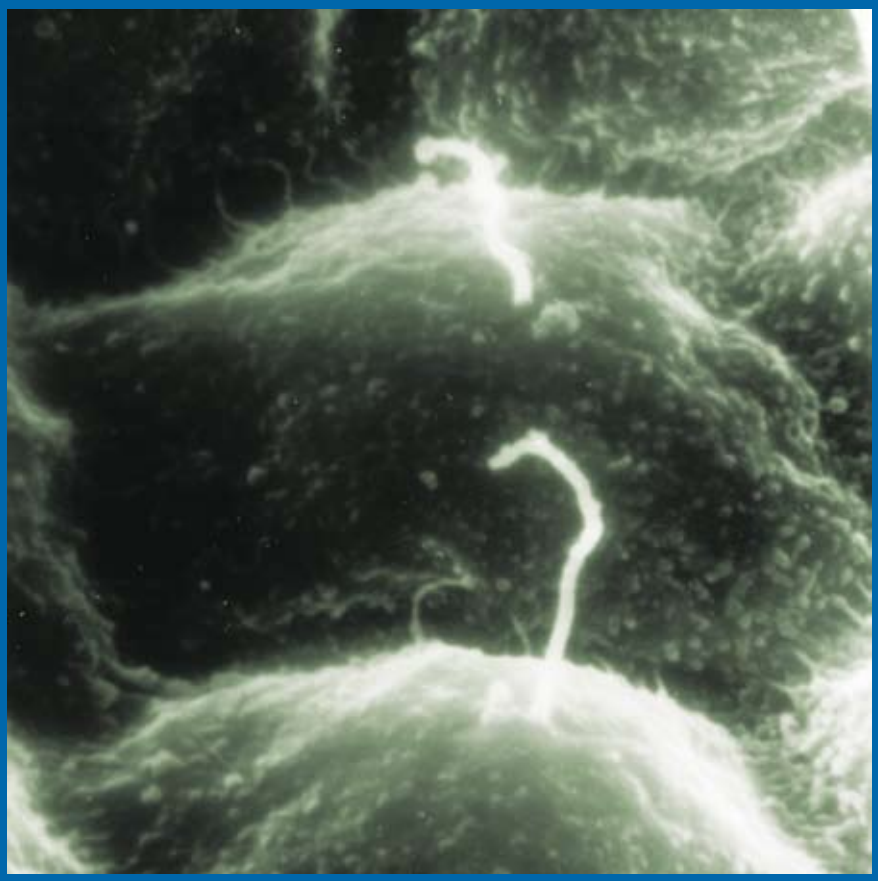
You could get hepatitis by:

- Having sex with an infected person without using a condom;
- Sharing drug needles;
- Having a tattoo or body piercing done with dirty tools that were used on someone else;
- Getting pricked with a needle that has infected blood on it;
- Living with someone who has hepatitis B;
- Sharing a toothbrush or razor with an infected person;
- Traveling to countries where hepatitis B is common; or
- Receiving a blood transfusion in the 1980s or earlier, before effective screening of donor blood for the hepatitis B virus.

An infected woman can give hepatitis B to her baby at birth.

You CANNOT get hepatitis B by:

- Shaking hands with an infected person;
- Hugging an infected person; or
- Sitting next to an infected person.



A scanning electron micrograph showing cilia present on kidney tubule cells. Cilia are hair-like structures that sense and process signals located outside the cell. Recent research, including research described in this chapter, has shown that defects in the cilia found on these kidney tubule cells may contribute to the development of cystic kidney diseases.

Kidney, Urologic, and Hematologic Diseases

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

Normal, healthy kidneys process about 200 quarts of blood a day to filter out about two quarts of waste products and extra water from the blood, excreting them as urine. In people with chronic kidney disease, the function of these life-sustaining organs is impaired. Kidney disease may progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. At the close of 2005, more than 485,000 patients were receiving treatment for ESRD.[1] An estimated 26 million Americans suffer from chronic kidney disease.[2] The leading cause of kidney disease is diabetes, with hypertension (high blood pressure) the second-leading cause. If unchecked, the recent increases in obesity and type 2 diabetes in the U.S. will have grave implications in several years, as more people begin to develop kidney complications of diabetes.

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

The NIDDK supports a significant body of research aimed at increased understanding of the biology underlying chronic kidney disease. The Institute's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease; the underlying mechanisms leading to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Areas of focus include diseases that collectively account for more than half of all cases of treated ESRD. Of special interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related glomerular diseases, including IgA nephropathy and the hemolytic uremic syndrome. The Institute's National Kidney Disease Education Program is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure. It represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect men and women of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research effort includes basic, clinical, and epidemiologic research on the genitourinary (GU) tract. The NIDDK has supported studies in benign and noncancerous urologic disorders and diseases, including benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, pelvic floor disorders, congenital anomalies of the genitourinary tract, and sexual dysfunction.

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

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[1] *U.S. Renal Data System, USRDS 2007 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2007.*

[2] *Coresh J, et al: Prevalence of chronic kidney disease in the United States. JAMA 298: 2038-2047, 2007.*

gland—is a painful condition that accounts for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genital and urinary systems. To determine the greatest scientific opportunities for research in these areas, the NIDDK is nearing completion of a Prostate Basic and Clinical Science Strategic Planning effort, which will serve as a guide for future scientific inquiry. The NIDDK is committed to enhancing research to understand, treat, and prevent these common and troubling disorders.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). NIDDK research includes both basic and clinical projects aimed at understanding UTIs and finding ways to prevent their recurrence. Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating, chronic, and painful bladder disease. The number of individuals suffering with IC/PBS is not known with certainty, but it has been estimated that 1.3 million adults in the U.S. may have the disorder, with more women affected (90 percent) than men.[3] NIDDK-supported basic and clinical research is focused on elucidating the cause(s) of IC/PBS, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. The NIDDK sponsors the Interstitial Cystitis Clinical Trials Group/Research Network to conduct clinical studies in IC/PBS. A new initiative, "Multi-disciplinary Approach to Chronic Pelvic Pain (MAPP)," is addressing many of the unanswered questions that impede research progress in both IC/PBS and chronic prostatitis, which share similar symptoms.

A conservative estimate is that approximately 12-13 million Americans, most of them women, suffer from urinary incontinence.[4,5] Many who have the disorder suffer in silence due to embarrassment and lack of knowledge about options available. The clinical field of urinary incontinence has changed dramatically in the last decade with the advent of new surgical procedures that have rapidly been introduced into the field. The NIDDK's Urinary Incontinence Treatment Network (UITN) has recently reported findings of the SISTEr trial (see research advances later in this section), which compared two surgical treatments for urinary incontinence. The Network is near completion of a second trial examining the effect of emptying the bladder on a regular schedule, along with Kegel exercises to strengthen pelvic muscles, to determine whether these practices will allow women to stop drug therapy and maintain the same level of bladder control. The third UITN study is recruiting patients to compare two minimally invasive surgeries for the treatment of stress urinary incontinence.

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

One of the most common causes of kidney failure in children is vesicoureteral reflux. In fact, abnormalities of the genitourinary tract are among the most common birth defects. The NIDDK is conducting a clinical trial to determine if the current practice of long-term antibiotics is necessary for the treatment of these children.

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

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[3] *Clemins JQ, et al: Interstitial Cystitis and Painful Bladder Syndrome in* Urological Diseases in America *(pp. 125-154). NIDDK, NIH Publication Number 07-5512, 2007.*

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

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

the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

PREVENTING URINARY TRACT INFECTIONS

Vaccination May Help Prevent Recurrent Urinary Tract Infections in Women: Urinary tract infections (UTIs) are extremely common and can interfere with work and daily activities. Women are highly susceptible to UTIs, and many women suffer from recurrent infections—sometimes three or more per year. Most are caused by the bacterium, *E. coli*. In a recent clinical trial, researchers found that a vaccine approach may be effective at preventing recurrent *E. coli* UTIs in women. In the trial, adult women with a history of frequent UTIs were randomly assigned to one of three treatment groups. One group received placebos only, one group received primary vaccinations, and one group received primary vaccinations plus additional monthly vaccine "boosters." The vaccine was designed to build up the body's immune defenses in the urogenital tract, where UTI pathogens take hold. The team found that vaccination was most effective against *E. coli* UTIs when vaccine boosters were provided. Almost 73 percent of vaccinated women who received boosters remained infection free 160 days after starting treatment, versus only 30 percent of those on placebos. The vaccine plus boosters regimen was especially effective at slowing the rate of *E. coli* UTI recurrence among sexually active women, and among women under the age of 52. Currently, women are prescribed antibiotics to prevent UTI recurrences, but drug-resistant UTI bacteria are increasingly making this approach less effective. The encouraging results of this trial suggest that a vaccination strategy targeting the urogenital tract may prove a good alternative approach to prevent recurrent UTIs in women.

Hopkins WJ, Elkahwaji J, Beierle LM, Leverson GE, and Uehling DT: Vaginal mucosal vaccine for recurrent urinary tract infections in women: results of a phase 2 clinical trial. J Urol 177: 1349-1353, 2007.

NEW TREATMENT FOR HYPEROXALURIA

Study Offers New Hope for Patients with Primary Hyperoxaluria Type 1: Intestinal bacteria show promise as a therapy for primary hyperoxaluria type 1, a rare condition characterized by the accumulation of oxalate due to deficiency of an enzyme that degrades this substance. In the kidneys, the excess oxalate combines with calcium to form calcium oxalate, a hard compound that is the main component of kidney stones. Deposits of calcium oxalate can lead to kidney damage and ultimately failure, and injury to other organs. Current therapeutic options for primary hyperoxaluria are few and not effective in a majority of patients. Researchers have now found that a number of intestinal bacteria, including *Oxalobacter formigenes (O. formigenes)*, can degrade oxalate. Building on this finding, a recent pilot study investigated whether oral administration of *O. formigenes* to primary hyperoxaluria patients for 4 weeks can reduce oxalate levels in blood and urine. The study demonstrated potential promise because three of five patients with normal kidney function showed decreased oxalate in their urine. In addition, two patients with kidney failure showed a significant drop in blood oxalate levels, as well as an improvement in their symptoms. The scientists found that this treatment strategy is safe and effective in reducing urine and plasma oxalate and thus, may be a useful therapeutic option for primary hyperoxaluria.

Hoppe B, Beck B, Gatter N, von Unruh G, Tischer A, Hesse A, Laube N, Kaul P, and Sidhu H: Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1. Kidney Int 70: 1305-1311, 2006.

GENETICS OF KIDNEY DISEASE

Novel Gene Implicated in Early-Onset Kidney Disease: Scientists have recently identified a role for the phospholipase C epsilon gene (*PLCE1*) in early-onset nephrotic syndrome. Nephrotic syndrome is a kidney disease characterized by elevated levels of protein in the urine, diminished levels of protein in the blood, and fluid retention and tissue swelling. Abnormal function of podocytes, specialized cells within the kidneys' filtering units, appears to be at the center of nephrotic syndrome. Widespread scarring of the kidney in this syndrome can result in diminished kidney function and end-stage renal disease, in which case dialysis or a kidney transplant is required. There is no effective long-term treatment.

At least six genes have previously been implicated in nephrotic syndrome. Researchers performed genome-wide scans of several extended families with nephrotic syndrome who did not carry any of the previously described mutations, to search for additional genetic clues to this condition. Seven families or individuals were found to carry mutations in their *PLCE1* gene. In six, the mutations resulted in a truncated protein; in the seventh, it produced a full-length but defective protein.

In the kidney, PLCE1 protein initiates a cascade of intracellular signaling events resulting in changes in gene expression, cell growth, and differentiation. Using cultured cells and tissue analysis, the researchers demonstrated that PLCE1 protein is expressed in normal podocytes. When PLCE1 protein expression was experimentally reduced during development in zebrafish, the kidneys showed anatomic changes consistent with nephrotic syndrome.

Some of the children in the study had been treated previously with steroids or the drug cyclosporine A, two drugs used to suppress the immune system. Most were not responsive to therapy, as are most patients with nephrotic syndrome. Surprisingly, two of them—now 6 and 13 years of age—retained near-normal kidney function. To explain this exception, the researchers hypothesize that PLCE1 protein may be necessary for the kidneys to complete a particular developmental phase, and that drug therapy somehow compensated for the absence of PLCE1 signaling during this critical window. Future studies will examine additional individuals with nephrotic syndrome to test these intriguing hypotheses, as well as characterize a recently-described mouse that lacks the *PLCE1* gene as a potentially valuable model system to study this condition.

Hinkes B, Wiggins RC, Gbadegesin R, Vlangos CN, Seelow D, Nürnberg G, Garg P, Verma R, Chaib H, Hoskins BE, Ashraf S, Becker C, Hennies HC, Goyal M, Wharram BL, Schachter AD, Mudumana S, Drummond I, Kerjaschki D, Waldherr R, Dietrich A, Ozaltin F, Bakkaloglu A, Cleper R, Basel-Vanagaite L, Pohl M, Griebel M, Tsygin AN, Soylu A, Müller D, Sorli CS, Bunney TD, Katan M, Liu J, Attanasio M, O'Toole JF, Hasselbacher K, Mucha B, Otto EA, Airik R, Kispert A, Kelley GG, Smrcka AV, Gudermann T, Holzman LB, Nürnberg P, and Hildebrandt F: Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. Nat Genet 38: 1397-1405, 2006.

Identification of New Genetic Cause of Kidney Disease: Researchers have identified a gene that, when mutated, leads to an inherited form of kidney disease known as nephronophthisis (NPHP) and may explain why kidney size is significantly decreased in this disease. Characterized by kidney degeneration during childhood, NPHP leads to renal failure and ultimately the need for kidney transplantation. By conducting a genome-wide scan of three related children with early onset NPHP and their family members, researchers identified a single mutation in the gene encoding GLIS2 that is linked to the disease. To gain insight into its functional role, the scientists studied GLIS2 in normal mice and in mice containing a mutated form of the protein. The GLIS2 protein is expressed in normal adult mouse kidney, specifically in the cilia—microscopic hair-like projections on the cell surface. When the *GLIS2* gene is mutated in mice, researchers observed that the kidneys demonstrated several of the hallmarks of NPHP, including fibrosis, smaller kidney size, and loss of tissue organization.

To understand the mechanism by which the diseased kidneys are decreased in size in NPHP, the researchers looked for genes that were specifically switched on in the mice lacking functional GLIS2. The researchers examined genes with a specific DNA sequence to which GLIS2 is known to bind, indicating that these genes are directly regulated by GLIS2. They found that the absence of GLIS2 resulted in greater expression of genes involved in such processes as change in cell type, cell signaling, fibrosis, and cell death. Notably, the activated genes indicated a high level of cell death in the diseased kidney without the cell proliferation necessary to maintain organ size. By discovering how GLIS2 regulates genes involved in a change in kidney cell type to one that is typically involved in fibrosis, the researchers may have uncovered an explanation for the loss of normal kidney structure and progressive fibrosis that occur in NPHP. These results provide insight into the cause of the reduced kidney size seen in NPHP and may guide future approaches to treating and preventing this inherited form of kidney disease.

Attanasio M, Uhlenhaut NH, Sousa VH, O'Toole JF, Otto E, Anlag K, Klugmann C, Treier AC, Helou J, Sayer JA, Seelow D,

Nürnberg G, Becker C, Chudley AE, Nürnberg P, Hildebrandt F, and Treier M: Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. Nat Genet 39: 1018-1024, 2007.

NEW INSIGHTS ON THE ROLE OF CILIA IN DISEASE

New Role of Cilia in Health and Disease: Recent research implicates the loss of cilia function in kidney disease and obesity. Cilia on many cell types are involved in cellular movement and, with stationary cells, as sensors that detect movement of fluids over the cells and detect molecules in the fluids. In the mouse kidney, this movement and its subsequent cellular signal are required for normal development of the organ. Researchers recently examined the effects of mutating two genes, *Tg737* and *Kif3a*, required for the formation and maintenance of cilia in adult mice. Surprisingly, loss of cilia throughout the body of the mouse did not lead to any immediate effects on the kidney, liver, or pancreas. Cysts eventually developed in the kidney and liver after 6 months, indicating that the cyst formation was not directly due to the inability of the cilia to convert sensory input into a cellular signal. While studying this mouse model of kidney disease, the scientists observed that these mutants exhibited a significant increase in their body weight that was not seen in control mice. This increase in body weight appeared to be primarily due to the fact that the mice ate a greater quantity of food than is optimal. The researchers also observed corresponding significant increases in fat mass and percentage of body fat, as well as elevated levels of molecules that act as satiety signals. To examine the underlying mechanisms of these effects, the researchers specifically deleted the cilia on neuronal cells in the hypothalamus section of the brain. These mice also became obese and demonstrated many of the same symptoms, suggesting that cilia on these cells are critical for regulating normal feeding behavior. Since the resulting obesity and related symptoms seen in mice resemble those seen in humans with insulin resistance and diabetes, this finding may not only provide significant insight into regulating feeding behavior and sensing "fullness," but may also provide a new target for therapeutic approaches for diabetes and obesity.

Davenport JR, Watts AJ, Roper VC, Croyle MJ, van Groen T, Wyss JM, Nagy TR, Kesterson RA, and Yoder BK: Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. Curr Biol 17: 1586-1594, 2007.

A Common Defect Links Multiple Disorders: Studies of families with two rare disorders have revealed the importance of genes that control the function of cilia. In a recent report, scientists conducted analyses to determine the variation across the human genome of families affected by cerebello-oculo-renal syndrome and Meckel syndrome. Individuals with these syndromes suffer from developmental problems, as well as from kidney cysts and liver fibrosis. In performing these scans, the researchers identified mutations in the gene *RPGRIP1L*, which codes for a protein component of the cilia. Additional experiments determined that *Rpgrip1l* is expressed in many organs during development. This finding is consistent with the multiple organs affected in patients with these two syndromes. Interestingly, *Rpgrip1l* is not the first cilia-associated gene to be associated with developmental problems. This observation suggests that disruption of cilia signaling can give rise to more generalized defects in development. While the precise biological function of *Rpgrip1l* is not known, its identification suggests that this signaling pathway may play a more central role in a number of diseases than previously realized.

Delous M, Baala L, Salomon R, Laclef C, Vierkotten J, Tory K, Golzio C, Lacoste T, Besse L, Ozilou C, Moutkine I, Hellman NE, Anselme I, Silbermann F, Vesque C, Gerhardt C, Rattenberry E, Wolf MTF, Gubler MC, Martinovic J, Encha-Razavi F, Boddaert N, Gonzales M, Macher MA, Nivet H, Champion G, Berthélémé JP, Niaudet P, McDonald F, Hildebrandt F, Johnson CA, Vekemans M, Antignac C, Rüther U, Schneider-Maunoury S, Attié-Bitach T, and Saunier S: The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. Nat Genet 39: 875-881, 2007.

DIETARY FACTORS AND THE RISK OF DEVELOPING KIDNEY STONES

Dietary Oxalate Is Not a Major Contributor to Kidney Stone Formation: Researchers have made the surprising discovery that increased intake of dietary oxalate does not substantially increase risk of kidney stones. A large percentage of kidney stones contain a compound called calcium oxalate. Since calcium and oxalate are both components of a normal diet, it

would be reasonable to propose that increased dietary intake of these compounds may increase the risk of developing kidney stones. To examine the link between oxalate intake and kidney stones, scientists analyzed historic dietary information from over 240,000 men and women participating in three major national long-term health studies. This information was compared to new patient complaints that were found to be associated with the presence of kidney stones. Quantifying dietary oxalate is difficult because of the lack of information about the oxalate content of most foods. Therefore, the researchers first examined oxalate intake using a measure of the 10 most common foods that contribute to dietary oxalate. They subsequently analyzed spinach intake alone as a measure of dietary oxalate, because raw or cooked spinach accounted for nearly half of the oxalate intake of the participants. The results of these analyses demonstrated that even those individuals who ate the highest levels of oxalate-containing foods or spinach had only a modestly increased risk of developing kidney stones compared to those individuals who ate little or no oxalate-containing foods or spinach. These results are quite surprising for patients, as well as for health care providers who treat kidney stones. Many physicians currently advise patients with kidney stones to decrease their intake of oxalate-containing foods. However, the results of this research suggest that decreasing oxalate intake in the diet may not be an effective strategy for preventing stone formation. For more information on the association between dietary factors and kidney stone formation, please see the "Scientific Presentation" in this chapter.

Taylor EN and Curhan GC: Oxalate intake and the risk for nephrolithiasis. J Am Soc Nephrol 18: 2198-2204, 2007.

SLOWING PROGRESSION OF POLYCYSTIC KIDNEY DISEASE

Targeting Cellular Pathway To Slow the Progression of Polycystic Kidney Disease: Using a rat model of autosomal dominant polycystic kidney disease (PKD), researchers have found that targeting vascular endothelial growth factor receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2) may slow formation of cysts and preserve kidney function. VEGFR1 and VEGFR2 were first discovered on the surface of endothelial (blood vessel) cells and have been shown to enhance cell division when stimulated. These receptors also play a role in cancer, by promoting tumor-associated blood vessel formation. They also are found on the surface of cancer cells, where they can stimulate cell division. The discoveries in cancer have led to the development of multiple agents designed to block VEGFR1 and VEGFR2 pathways.

Scientists interested in PKD took advantage of the recent discovery of VEGFR1 and VEGFR2 on the surface of renal tubular epithelial cells, as well as the agents designed to target these receptors to explore the role of VEGFR1 and VEGFR2 in the development of cysts and in kidney function. Using methods to decrease the levels of VEGFR1 and VEGFR2 on the surface of the renal tubular epithelial cells, the researchers showed that the division of epithelial cells in existing cysts was decreased, leading to delayed growth of cysts. Blocking VEGFR1 and VEGFR2 also decreased serum creatine and blood urea nitrogen—two important indicators of kidney function—indicating that this treatment may improve kidney function. Furthermore, the specific methods used to block VEGFR1 and VEGFR2 did not lead to measurable negative consequences for kidney function, complications that have been seen with other agents used to target this pathway. The report of the VEGFR1 and VEGFR2 studies is exciting for patients with PKD because it provides the impetus for further exploration of the contribution of these receptors to PKD, which could have significant implications for how this disease is treated in the future. Current treatment of PKD focuses mainly on detecting and monitoring cysts and managing patients' blood pressure. The VEGFR1 and VEGFR2 results suggest that effective targeted therapies could be developed to treat PKD.

Tao Y, Kim J, Yin Y, Zafar I, Falk S, He Z, Faubel S, Schrier RW, and Edelstein CL: VEGF receptor inhibition slows the progression of polycystic kidney disease. Kidney Int 72: 1358-1366, 2007.

NOVEL CAUSE OF IRON OVERLOAD IN THALASSEMIA DISORDERS

Study Identifies Protein That Contributes to Iron Overload in Thalassemias: NIDDK intramural scientists have discovered a novel cause of iron

overload in patients with thalassemia. This inherited blood disease is characterized by impaired production of hemoglobin—the oxygen-carrying protein in red blood cells—and by anemia. The scientists found that thalassemia patients had unusually high levels of the protein growth differentiation factor 15 (GDF15) in their blood when compared to normal volunteers. From subsequent experiments, it appears that GDF15 suppresses the production of the liver protein hepcidin, an important regulator of iron metabolism in the body. A drop in hepcidin levels leads to an increase in the uptake of dietary iron in the gut. The body lacks a mechanism to excrete excess iron. Too much iron can cause damage to many organs, including the heart and liver. The identification of GDF15 as an important contributor to the regulation of normal iron levels has implications for the management of iron metabolism in patients with thalassemia and other diseases. It may also be a valuable tool in the development of future therapies for these health problems.

Tanno T, Bhanu NV, Oneal PA, Goh S-H, Staker P, Lee YT, Moroney JW, Reed CH, Luban NLC, Wang R-H, Eling TE, Childs R, Ganz T, Leitman SF, Fucharoen S, and Miller JL: High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med 13: 1096-1101, 2007.

New Insights on Bladder Control in Women

Loss of bladder control, or urinary incontinence, can be embarrassing and difficult to deal with. It is also a common and costly condition that reduces quality of life for many Americans, especially women. According to the NIDDK's *Urologic Diseases in America* project, up to three-fourths of women have some degree of incontinence. It is estimated that, in 2000, the direct cost of incontinence for women—including hospital stays and visits to office-based physicians, hospital outpatient clinics, and emergency rooms—was $453 million.[1] These women usually have stress or urge urinary incontinence—the most common forms of incontinence—and sometimes a combination of both.

Now, in the largest and most rigorous U.S. trial comparing two traditional operations for stress urinary incontinence in women, a team of urologists and urogynecologists supported by the NIDDK, the National Institute of Child Health and Human Development, and the NIH Office of Research on Women's Health, has found that a sling procedure helps more women achieve dryness than the Burch technique. The results of the trial will help women with stress incontinence and their doctors make better-informed choices based on clear benefits, risks and personal preferences.

SISTEr Results

Stress urinary incontinence, in which coughing, laughing, sneezing, running or lifting heavy objects causes urine to leak, is sometimes treated with surgery designed to provide additional support to the bladder neck and urethra during increases in abdominal pressure that occur with these kinds of activities. Many different surgical procedures exist to treat stress urinary incontinence. However, randomized, controlled trials comparing these operations for safety and efficacy are rare.

The Stress Incontinence Surgical Treatment Efficacy Trial (SISTEr) randomly assigned 655 women with either pure stress urinary incontinence or a combination of stress and urge incontinence to receive the sling or Burch procedures. Complete information on measures used to assess urinary incontinence was available for 520 participants 24 months after surgery. Quality of life, patient satisfaction and side effects were also studied. SISTEr found that significantly more women with a sling made from the patient's own tissue and placed around the urethra for additional support were dry, compared to women with a Burch colposuspension, in which sutures are attached to a pelvic ligament to support the urethra. Two years after surgery, 47 percent of women who had the sling procedure and 38 percent who had a Burch were dry overall, including leakage that could have been caused by urge incontinence. Considering only stress-specific leakage, 66 percent of women with a sling and 49 percent with a Burch procedure were dry.

While most women in the study were satisfied with the results of treatment, those with a sling were significantly more satisfied. Eighty-six percent with a sling were satisfied, compared to 78 percent of the Burch group. However, side effects were more common among women with slings, tempering the positive results of the procedure. The most common side effect was urinary tract infections, which occurred in 63 percent of women with a sling and 47 percent of the Burch group. Women with a sling also had more voiding problems (14 percent versus 2 percent) and persistent urge incontinence, the loss of urine just before feeling a strong, sudden urge to empty the bladder (27 percent versus 20 percent). Nineteen women with slings who had difficulty voiding after treatment needed surgery to correct the problem; none in the Burch group needed corrective surgery for voiding problems.

Studies predating SISTEr were small, short-term, or less stringent about diagnostic criteria and outcome measures, producing inconsistent results across studies. SISTEr set a higher bar by standardizing definitions, clinical evaluations, and surgical procedures at all sites and by using "composite outcome measures and a more rigid definition of success compared to other studies," according to the study.

SISTEr defined two levels of treatment success. Success specific to stress incontinence required that women have no symptoms of leakage during physical activities, no leakage during a relevant stress-test, and no re-treatment for the problem. Overall success required that women

meet stress-incontinence-specific treatment goals, have a negative pad test and have no leakage episodes recorded on a 3-day voiding diary. This higher bar may account for lower success rates observed in SISTEr than in earlier trials. However, this carefully conducted trial will inform the planning of future trials of surgical therapies for urinary incontinence and other urological health problems.

The Urinary Incontinence Treatment Network

SISTEr is the first trial completed by the Urinary Incontinence Treatment Network (UITN), a multi-center research network supported by the NIDDK. Beginning in 2000, the NIDDK, the National Institute of Child Health and Human Development, and the NIH Office of Research on Women's Health established this Network to conduct a series of rigorous, long-term trials of common incontinence therapies in women. The UITN is also collecting data on body weight and diabetes, which could serve as a resource for ancillary studies to investigate the association of urinary incontinence with obesity and diabetes. In addition to SISTEr, two other UITN studies are in the wings:

- *BE-DRI:* Behavior Enhances Drug Reduction of Incontinence (BE-DRI) is a trial of therapies for urge incontinence. BE-DRI asked 307 women to make changes such as emptying the bladder on a regular schedule and to practice Kegel exercises to strengthen pelvic muscles to learn if these common treatments would allow women to stop drug therapy and maintain the same degree of bladder control. The BE-DRI trial has been completed and as this document goes to press, trial results are expected to be published shortly.

- *TOMUS:* The third UITN study, the Trial of Mid-Urethral Slings (TOMUS), is recruiting patients to compare two minimally invasive surgeries for the treatment of stress urinary incontinence. Both procedures include placement of a synthetic mesh sling (rather than a sling made from the patient's own tissue, as was used in SISTEr), and have been approved by the Food and Drug Administration for treatment of stress incontinence. For a list of centers enrolling patients for TOMUS, visit *http://www.uitn.net* or search for TOMUS at *http://www.clinicaltrials.gov*

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[1] *Litwin MS and Saigal CS: Introduction in* Urologic Diseases in America *(pp. 1-7). NIDDK, NIH Publication Number 07–5512, 2007.*

Urologic Diseases in America

Urologic Diseases in America, released in Spring 2007, was developed by a team of epidemiologists, health economists, statisticians, programmers, and urologists—with NIDDK funding. According to the report, bladder, prostate and other urinary tract diseases cost Americans nearly $11 billion a year and Medicare's share of these expenses exceeds $5.4 billion. The report describes more than a dozen diseases of children and adults, among them congenital abnormalities, erectile dysfunction, chronic prostatitis, interstitial cystitis, urinary incontinence and a chapter on the topic of sexually transmitted diseases, contributed by the Centers for Disease Control and Prevention. The findings include:

- *Urinary Tract Infections:* Medical care for almost 12.8 million urinary tract infections in women alone costs nearly $2.5 billion annually. Adding the cost for men raises the total to $3.5 billion; Medicare's share was $1.4 billion. Another $96.4 million was spent on 3.3 million prescriptions. More than half of all women will have an infection during their lifetimes. While only 20 percent of infections are in men, they are more often hospitalized, and are out of work about twice as long as women due to this health problem.

- *Kidney Stones:* While hospitalizations, length of stay and the need for open surgery are declining for kidney stones, medical care still costs $2.1 billion annually, with another $4 million to $14 million spent on prescription drugs. Men are two-to-three times more likely than women to develop a stone, but more people of all ages and races are now getting them: an estimated 5 percent of adults between 1988 and 1994, up from nearly 4 percent between 1976 and 1980. Compared to Caucasians, African Americans and Mexican Americans have a 70 percent and 35 percent lower risk, respectively, of developing a stone.

- *Childhood Urologic Diseases:* Although data for childhood urologic diseases are scarce, urinary problems in children cost at least $75 million dollars a year. Vesicoureteral reflux, the abnormal flow of urine from the bladder up toward the kidneys, affects about 10 percent of all children and makes them prone to urinary tract infections and kidney damage. The cost of hospitalizations for reflux alone rose from $10 million in 1997 to $47 million in 2000; Southern states, defined using U.S. Census Bureau regions, saw the highest rise—56 percent—attributable to a doubling in the number of cases.

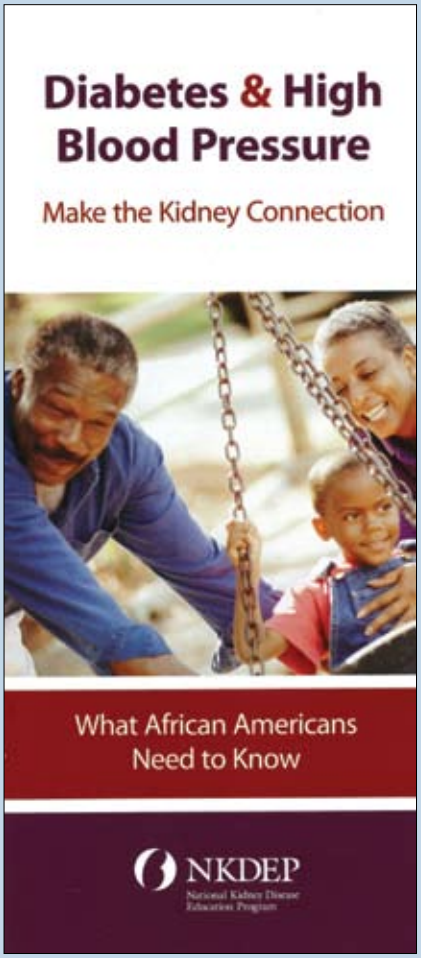
Urologic Diseases in America—printed books and CDs—may be ordered from the National Kidney and Urologic Diseases Information Clearinghouse at 1–800–891–5390, *nkudic@info.niddk.nih.gov*, and at *www.catalog.niddk.nih.gov*

National Kidney Disease Education Program: New Publication Helps African Americans "Make the Kidney Connection"

An estimated 26 million Americans suffer from chronic kidney disease (CKD)[1] and, according to the NIDDK-supported United States Renal Data System (USRDS), more than 340,000 patients are on dialysis.[2] Patients with CKD are at increased risk for kidney failure. It is estimated that treating the number of people with kidney failure, also called end-stage renal disease (ESRD), through dialysis or kidney transplantation costs the U.S. health care system more than $30 billion every year.[2] ESRD is an enormous public health problem that disproportionately affects minority populations.

Helping address these issues is the NIDDK's National Kidney Disease Education Program (NKDEP). This educational program seeks to raise awareness of the seriousness of kidney disease, the importance of testing those at high risk—those with diabetes, high blood pressure, cardiovascular disease, or a family history of kidney disease—and the availability of treatment to prevent or slow kidney failure. The progression from CKD to kidney failure can be prevented or delayed if it is detected and treated early enough. The NKDEP underscores that effective treatments and management strategies for kidney disease exist, yet are being underutilized.

Recently, the NKDEP created an educational brochure tailored specifically for African Americans at risk for kidney disease. The brochure explains the connection between diabetes, high blood pressure, and kidney disease. The brochure also encourages those at risk to talk to their health care providers about getting tested.



This new NKDEP brochure tailored for African Americans at risk for kidney disease explains the connection between diabetes, high blood pressure, and kidney disease.

African Americans are disproportionately affected by kidney failure due in part to higher rates of diabetes and high blood pressure—the two leading causes of kidney failure. In a press release announcing the new publication, Dr. Griffin Rodgers, NIDDK Director, noted that, "Diabetes and high blood pressure are all too common among African Americans, yet many are unaware of their risk factors and the importance of getting tested. NKDEP recognizes the importance of promoting key messages about kidney disease risk factors to this audience." Dr. Andrew Narva, NKDEP Director, added, "Unlike many diseases, kidney disease often has no symptoms until it is very advanced. For this reason and others, it is important for African Americans to not only become aware of their risk, but also to learn about the steps they can take to keep their kidneys healthier longer. An important step is to get tested."

The brochure explains the blood and urine tests used to detect kidney disease in simple, easy-to-read language. It also outlines several steps that African Americans can take to protect their kidneys. These steps include:

- Keeping kidneys healthy by managing diabetes and high blood pressure;
- Asking health care providers to test blood and urine for kidney disease; and
- Talking to health care providers about treatment options if already diagnosed with kidney disease.

In developing the brochure, the NKDEP worked with health care professionals who routinely care for African American patients at risk for kidney disease. Reviewers included NKDEP Coordinating Panel members and representatives from the Association of Minority Nephrologists. By partnering with national, state, and local organizations, including government agencies, NKDEP hopes to reach a large number of African Americans with this information.

For more information about the brochure and other NKDEP materials, visit *www.nkdep.nih.gov* or call 1-866-4 KIDNEY (1-866-454-3639).

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[1] Coresh J, et al: Prevalence of chronic kidney disease in the United States. JAMA 298: 2038-2047, 2007.

[2] www.usrds.org/2007/pdf/00a_precis_07.pdf

STORY OF DISCOVERY

Polycystic Kidney Disease

Polycystic kidney disease, or PKD, is the fourth leading cause of kidney failure in the U.S.[1] Fluid-filled cysts form in the kidneys and other organs and can, as they grow over time, compromise kidney function. Patients with the disease typically have high blood pressure, urinary tract infections, and chronic pain. There is no primary treatment for PKD, and patients generally receive drugs to control their blood pressure and manage their pain. However, knowledge of the causes of PKD has increased dramatically in the past 20 years due to NIDDK-supported research. Scientists have a better understanding of the genetic causes of PKD, and are studying the use of new technologies to improve disease detection and monitoring. Because of the efforts of many dedicated scientists, there is hope for the future for people with PKD and their families.

What is Polycystic Kidney Disease?

Polycystic kidney disease (PKD) is a genetic disorder characterized by the growth of numerous fluid-filled cysts. These cysts develop primarily in the kidneys, but also can appear in organs such as the liver, pancreas, spleen, and thyroid. In the kidneys, these cysts can slowly replace much of the mass of the kidneys, reducing kidney function and leading to kidney failure. About half of people with the most common form of PKD progress to irreversible kidney failure, also called end-stage renal disease (ESRD). When this occurs, usually in the fifth or sixth decade of life, patients require either a kidney transplant or dialysis to survive. In the United States, an estimated 600,000 people have PKD, and it is the fourth leading cause of kidney failure. While there is no effective treatment for the underlying causes of PKD, patients are usually prescribed pain-relieving drugs, antibiotics to treat infections, as well as medications to control blood pressure that are aimed at preserving or slowing the decline in kidney function.

There are two major inherited forms of PKD, called autosomal dominant and autosomal recessive. Autosomal Dominant PKD, or ADPKD, accounts for about 90 percent of all cases. People with ADPKD usually develop symptoms between the ages of 30 and 40, but symptoms can appear earlier, even in childhood. The genetically recessive form of PKD, Autosomal Recessive PKD, or ARPKD, is a rare inherited form of the disease that displays symptoms in the earliest months of life, even in the womb.

Past Treatment for PKD

About 30 years ago, knowledge about the causes and progression of PKD was limited. The details of the genetics of the dominant form of PKD were unknown. Doctors knew that, on average, half of children born to an affected parent would develop the disease, and that it could be transmitted by either the mother or the father. The mechanism by which the disease caused cysts to form and grow in the kidneys was not known. Diagnosis of well-established disease in adults was relatively straightforward using the imaging techniques that were available at the time, such as ultrasound. However, diagnosis of earlier stages of disease in children and young adults was much more difficult. By the time most people were diagnosed, their kidneys were so damaged that kidney function had begun to decline.

Treatment options for people with chronic kidney disease in general, and ADPKD in particular, were also inadequate. No specific therapy was available. The importance of controlling blood pressure and dietary protein intake in patients with chronic kidney disease was not recognized. Two life-saving kidney function replacement therapies—hemodialysis and kidney transplantation—were developed through fundamental NIH research in the 1960s. Although they were increasingly available, neither was ideal.

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Genetic Underpinnings of PKD and Insights from Animal Models

The emergence of molecular biology and modern biotechnology in the late 1970s and early 1980s permitted researchers for the first time to examine in detail the genetic underpinnings of a number of diseases. Scientists have identified two genes associated with ADPKD. The first was found in 1985 on chromosome 16 and was named *PKD1*. The second gene, *PKD2*, was localized to chromosome 4 in 1993. Within 3 years, scientists had isolated the proteins these two genes produce—polycystin-1 and polycystin-2. Most cases of the dominant form of PKD can be traced back to mutations in one of these two genes. However, evidence suggests that the disease development also requires other factors. Normally, polycystin-1 and polycystin-2 form an ion channel on the surface of kidney cells. This channel regulates the flow of calcium into and out of the cell. Mutation of either gene inhibits the activity of the channel, thus disrupting calcium-dependent intracellular signaling pathways.

This ion channel is part of a complex of proteins located on the cell surface at the site where tiny, hair-like projections called cilia emerge from the cell into the renal tubule, where waste products are filtered into what will become urine. Under normal conditions, the cilia on the surface of these renal tubule cells detect changes in urine flow, and transmit this information inside the cell through the activation of various molecular signaling pathways. One signaling mechanism is the opening of the ion channel formed by polycystin-1 and -2. The opening allows calcium ions to enter the cell, setting off a cascade of signaling events. However, when one or both are mutated, the channel does not function properly. As a result, calcium does not enter the cell, and the metabolic response to changes in urine flow is disrupted. This abnormality in calcium signaling may result in cells that grow abnormally and retain fluid, ultimately giving rise to multiple, fluid-filled cysts characteristic of PKD.

Disruptions in cilia signaling have been found to underlie a number of diseases of the kidney, as well as other organs. Many genes encode proteins that localize to the cilia, and mutations in these genes often produce similar clinical manifestations. These observations have given rise to the hypothesis that many cystic kidney diseases may arise from defects in primary cilia signaling. Future efforts will be devoted to improved understanding of cilia signaling and identifying potential new therapeutic targets. Because cilia are found on the surface of almost all cells in the body, insights gained from these studies may also benefit people suffering from a number of diseases in which cilia signaling is impaired.

Researchers have also identified the gene associated with ARPKD, called *PKHD1*. The protein encoded by this gene, known as fibrocystin or polyductin, is present in fetal and adult kidney cells, and is also present at low levels in the liver and pancreas. Its precise biological function is unknown.

Current Clinical Management and Research Studies of PKD

Advances in knowledge about cyst formation and disease progression have been complemented by improvements in the early detection and treatment of the most common form of PKD. The NIDDK supports a number of clinical studies aimed at furthering our knowledge about the origins, progression, and optimal treatment of this disease.

The NIDDK-supported Consortium for Radiologic Imaging Studies of PKD (CRISP) was established to develop innovative imaging techniques and analyses to follow disease progression or to evaluate treatments for the common form of the disease. Importantly, the CRISP study demonstrated that magnetic resonance imaging (MRI) could accurately track structural changes in the kidneys, and that such methods may be able to predict functional changes earlier than standard blood and urine tests in people with the common form of PKD.

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The respective roles of *PKD1* and *PKD2* in disease progression, as indicated by ultrasound analysis, have remained unclear. The CRISP investigators, using a more sensitive MRI method, reported that patients with the *PKD1* gene have more cysts and significantly larger kidneys than those with the *PKD2* gene. Data from the CRISP study suggest that this difference results from earlier development of cysts, not from a faster growth of cysts, in patients with *PKD1* mutations. These clinically important results will inform the development of targeted therapies for patients with this form of the disease.

To expand and follow-up on the important insights gained in the CRISP study, the NIDDK has funded an extension, CRISP II, to continue to monitor this valuable cohort of patients. The extension will enable researchers to determine the extent to which changes in kidney volume do in fact predict changes in kidney function.

The NIDDK, with co-funding from the PKD Foundation, is also conducting two clinical trials of people with the most common form of PKD—one in patients with early kidney disease and another in patients with more advanced disease. These two trials are the largest multi-center studies of PKD conducted to date, and are collectively termed HALT-PKD. These studies are testing whether optimum blood pressure management, in combination with drugs—either angiotensin-converting enzyme inhibitors or angiotensin receptor blockers—will slow the progression of this disease. The NIDDK is also funding an investigator-initiated interventional trial of optimum blood pressure therapy in children and young adults. These interventional studies are the first clinical trials to implement and formally validate the imaging surrogate marker of PKD progression that was developed by the CRISP study.

Hope for the Future

Investigators are continuing to pursue basic biologic studies of the causes of PKD, as well as new avenues for therapies, in the hope that diagnosis and treatment can be improved. As scientists' understanding of the genetics and progression increases, it is hoped that there will be a decrease in the number of patients with the disease who progress to ESRD. Because PKD can affect patients very differently, even within the same family, the NIH is assembling a large genetic sample collection for future investigations. Studies of these samples may help to identify genetic markers that might predict who will develop more rapidly progressive kidney disease. These genetic studies could also provide new information on identifying key disease pathways and aid in the design of new drug treatment strategies. The studies also could yield clues about how to intervene earlier, more precisely, and more effectively in these patients. Earlier intervention, more intensive management of high blood pressure, and use of drugs that target kidney fibrosis may delay progression to ESRD and give patients additional years of life without the need for dialysis or a kidney transplant. For patients who eventually do need dialysis, the NIH is conducting a trial to determine whether more frequent dialysis improves their quality of life.

Although there have been advances in the knowledge base about dialysis and improvements in technology, a functioning kidney transplant remains a patient's best hope of living a more normal life. However, normal life expectancy and health-related quality of life are rarely, if ever, restored by organ transplantation. Furthermore, despite the best immunosuppressive therapies, many patients with kidney transplants still lose their transplanted kidneys due to rejection of the transplant by the body's immune system. Better strategies to maintain the

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function of transplanted kidneys and prevent chronic scarring are likely to emerge from ongoing basic research and improved imaging methods. The NIDDK and NIH will continue to support research into kidney disease in general, and PKD in particular, working across Institutes and joining with other partners to better understand, monitor, and treat this disease.

[1] *U.S. Renal Data System, USRDS 2007 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2007.*

Kidney Stones as a Systemic Disease

Dr. Gary Curhan

Dr. Gary Curhan is an Associate Professor of Medicine at Harvard Medical School and an Associate Professor of Epidemiology at the Harvard School of Public Health. He is a leading researcher in the field of kidney disease, as well as a number of other chronic diseases, including interstitial cystitis and high blood pressure. The following represents highlights of a scientific presentation by Dr. Curhan at the September 2007 meeting of the NIDDK National Advisory Council. Dr. Curhan shared with the Council his view of kidney stone disease as an organ-specific manifestation of a more generalized systemic disorder, rather than simply a disease of the kidneys.

Kidney stone disease is a common and painful health problem in the U.S. It is also a growing problem: the number of people in the U.S. with kidney stones has increased significantly over the past 30 years. White Americans are more prone to develop kidney stones than African Americans, and men are more likely to develop stones than women. For unknown reasons, some individuals are prone to repeatedly developing stones. Each year, people make almost three million visits to health care providers and more than half a million people go to emergency rooms for kidney stone problems.[1]

The first symptom of a kidney stone typically appears when the stone moves from the kidney into the ureter, causing irritation or blockage and resulting in extreme pain. Most kidney stones can pass harmlessly—though not painlessly—through the urinary system. In such cases, medication to alleviate the pain may be the only medical intervention needed. Stones that cause lasting symptoms or other complications may be treated by various techniques, most of which do not involve major surgery. In severe cases, however, surgery may be required to remove the stone.

In addition to being extremely painful, kidney stones also are costly to treat. According to the 2007 edition of *Urologic Diseases in America*, kidney stones are the second-costliest urologic disease, accounting for over $2 billion spent on medical care,[2] with another $4 million to $14 million spent on prescription drugs.[3] These numbers do not include costs not associated with direct medical expenditures, such as time lost from work.

Composition of Kidney Stones

Kidney stones can consist of a number of different components. The most common type of stone, accounting for two-thirds of all stones, is a combination of calcium and oxalate. Less common types of stones include stones caused by urinary tract infections, and stones made of uric acid or the amino acid cystine. "Nephrolithiasis" is the medical term used to describe stones occurring in the kidney, while stones in the urinary tract are formally designated as "urolithiasis." For the sake of simplicity, "kidney stone" is often used to designate stones regardless of their location in the kidney or urinary tract.

Urine is a liquid with various substances dissolved in it, and there is a finite amount of material that can be dissolved in a given quantity of water. If this limit is exceeded, material will fall out of solution and crystallize. Once this process starts, the nascent crystals attract other dissolved elements in the water, and the crystal grows in size. Although the precise steps that lead to kidney stone formation are not known, one way to think about crystal formation is as a problem of too much material trying to remain dissolved in too little water.

SCIENTIFIC PRESENTATION

Risk factors for kidney stones encompass gastrointestinal, skeletal, and metabolic factors, as well as obesity. At first glance, the connection of most of these factors to the kidney may not be obvious. However, closer examination reveals clues that implicate kidney stone disease as an organ-specific manifestation of more general systemic disturbances.

Risk Factors: Gastrointestinal

Because the most common type of kidney stone, the calcium oxalate stone, consists of two components found in a normal diet, it might stand to reason that increasing consumption of these factors would increase the risk of stone formation. Indeed, patients prone to developing stones are often counseled to limit their dietary intake of calcium and oxalate. However, studies reveal that increased dietary intake of calcium does not increase the risk of stone formation and may in fact reduce the risk. Increased dietary intake of oxalate has, at best, a modest impact on stone risk. Surprisingly, however, increased dietary intake of fructose correlates with a dramatic increase in risk of developing a kidney stone. Fructose is one of the two sugar molecules that comprise ordinary table sugar and is a major component of high fructose corn syrup, which is used in a large number of food products. It seems that, in some individuals, fructose can be metabolized into oxalate. This observation underscores the complexity in using diet modification in people prone to developing kidney stones, because traditional advice to limit intake of oxalate-containing foods may not be sufficient to reduce risk of stone development (for more details regarding the assessment of dietary oxalate and its role in stone formation, see the accompanying research advance, "Dietary Oxalate Is Not a Major Contributor to Kidney Stone Formation").

Risk Factors: Bone

In addition to providing structural support and protecting internal organs, the skeleton represents a large repository for calcium. The skeleton is an active site of tissue breakdown and regeneration throughout life. In diseases such as osteoporosis, more calcium is lost from bone than is deposited, resulting in a net negative calcium balance. Researchers have known for years that patients with elevated calcium levels in their urine are likely to have lower bone mineral density, emphasizing that there are metabolic sources for urinary calcium, as well as dietary sources.

Of course, skeletal remodeling is not the only source of circulating calcium, as this mineral is an important component of the diet. Does increased calcium in the diet increase one's risk of developing kidney stones? Quite the opposite: several large epidemiologic studies and one randomized trial suggest that high dietary calcium intake is associated with a decreased risk of kidney stones, and that people with the lowest dietary calcium intake had an increased risk of kidney stones. The reason for this is unclear, but it is possible that higher levels of dietary calcium bind to oxalate in the digestive tract and prevent it from being absorbed and eventually moving to the kidneys where it might form stones.

Risk Factors: Obesity

It has been know for years that increasing body weight puts individuals at risk for high blood pressure, diabetes, and a host of other health problems. Recent research has also uncovered a role for obesity in the formation of kidney stones. Studies have shown that the risk of stone formation can be almost twice as great in women who weigh more than 220 pounds compared to those who weigh less than 150 pounds; overweight men are also at higher risk. The increase in relative risk is also seen if one looks at body mass index, which takes both height and weight into account. The reason for this correlation is unclear, but it is the subject of ongoing research.

As rates of obesity in the U.S. continue to rise, more people are turning to bariatric surgery as a way to address the problem. In this surgery, doctors alter the digestive tract in order to restrict food intake and, in some cases, interrupt the digestive process. When

researchers examined urine oxalate levels in patients who had undergone this procedure, they found levels were two to three times higher than normal, and elevation in oxalate seems to result in a higher risk of stone formation. This finding underscores the complex metabolic pathways that regulate oxalate absorption and excretion and how changes to the digestive tract may have unexpected results on overall metabolism.

Risk Factors: Endocrine and Metabolic Pathways

Diabetes significantly increases an individual's risk of kidney disease, blindness, amputation, and cardiovascular disease. Scientists have recently shown that diabetes also increases the risk of developing kidney stones between 20 and 50 percent, and that this increase is especially apparent in younger women. There is also evidence that people with diabetes have a lower than normal urine pH—meaning that their urine is more acidic. This change in urine composition, sometimes accompanied by a decrease in urine volume, may also contribute to stone formation in these individuals. This observation further emphasizes that kidney stones may arise as much from generalized metabolic derangement as from kidney-specific factors.

Risk Factors: Genetics

As is the case with many diseases, it is likely that kidney stones arise from both environmental and genetic causes. Much of what is known about stone formation concerns dietary and metabolic factors, but it is likely that genetics plays an important role in determining an individual's likelihood of developing kidney stones. Currently, a number of potential candidate genes have been identified, including the calcium sensing receptor, the vitamin D receptor, and the oxalate transporter protein in the intestine. Large genome-wide association scans that might identify other candidates have only recently begun, but hold great promise for the future (for more information about genome-wide association scans, see the chapter on Cross-Cutting Science.)

Conclusion

Kidney stone disease should be thought of as a systemic disorder and not just a disease of the kidneys. In the past several years, significant progress has been made in understanding the causes of the disease, but much work remains to be done. Moving forward, new paradigms regarding the underlying causes of the disease will shape the research agenda, especially regarding the origins of stones and the risk factors that contribute to their formation. Future large studies of genetics and gene-environment interactions will further our understanding of this complex disorder.

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[1] *Kidney Stones in Adults, National Kidney and Urologic Diseases Information Clearinghouse, National Institute of Diabetes and Digestive and Kidney Diseases. http://kidney.niddk.nih.gov/kudiseases/pubs/stonesadults/index.htm*

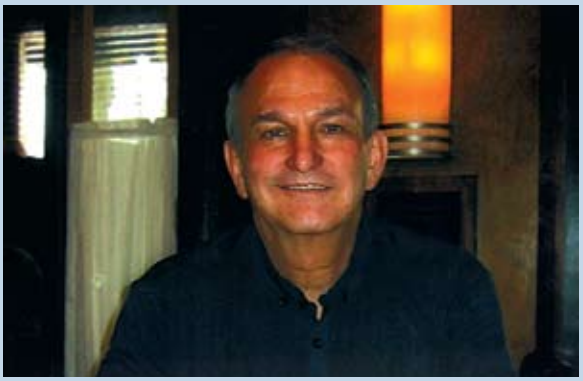
[2] *Litwin MS and Saigal CS: Introduction in* Urologic Diseases in America *(pp. 1-7). NIDDK, NIH Publication Number 07–5512, 2007.*

[3] *Pearle MS, Calhoun E, and Curhan GC: Urolithiasis in* Urologic Diseases in America *(pp. 281-319). NIDDK, NIH Publication Number 07-5512, 2007.*

PATIENT PROFILE

Richard Gordon

Study Finds Benign Prostatic Hyperplasia (BPH) in Older Men Effectively Treated with Drugs



Richard Gordon

"I couldn't get a good night's sleep," said 64-year-old corporate executive and grandfather of two, Richard Gordon. "I'd get up five to eight times a night to urinate. Also, I'd be sitting with friends at a Chicago Bulls basketball game with 2 minutes left in a close contest, the ball in Michael Jordan's hands—and I'd have to head to the men's room because of a sudden urge to urinate!" he said, adding that he's a big fan of spectator sports.

That was 15 years ago. Richard was suffering from a condition called benign prostatic hyperplasia, or BPH, which can manifest symptoms in men as young as 50. BPH is a condition in which the prostate gland enlarges, compressing the urethra and interfering with the normal flow of urine. Richard was experiencing frequent urination, a typical BPH symptom (see sidebar), and it was adversely affecting his sleep pattern and lifestyle.

Although surgical removal of the enlarged part of the prostate was considered an effective long-term solution for many patients with BPH, researchers continued to search for a way to shrink, slow, or stop the growth of the prostate without the use of invasive surgery, risks of which include possible incontinence and impotence.

Today, as a result of participating in a study called the Medical Therapy of Prostatic Symptoms (MTOPS) trial, sponsored by the NIDDK, Richard said that the drug regimen he was put on has his BPH under control. "I'd definitely recommend others take part in such studies," said Richard. "It has improved the quality of my life, and I feel I'm in a much better position to make informed decisions about how to treat BPH."

Medical Therapy of Prostatic Symptoms (MTOPS)

The MTOPS trial enrolled over 3,000 participants with symptomatic BPH from 1993 to 1998 and was conducted at 17 clinical sites in the U.S. The trial was designed to test whether two FDA-approved drugs—finasteride and doxazosin, alone or together—could prevent or reduce the progression of the symptoms of BPH. Finasteride is a 5-alpha reductase inhibitor, so named because it inhibits the action of the enzyme that converts the male hormone testosterone to its more potent derivative dihydrotestosterone (DHT). DHT is a key regulator of prostate growth. Doxazosin is an alpha-blocker, a drug that relaxes smooth muscle and, in the context of BPH, can allow urine to flow more freely through the urethra, and reduce the symptoms of urinary frequency and urgency. Both classes of drugs had been previously approved for treatment of BPH, but they had not been compared head-to-head in a long-term clinical trial, and they had not been formally tested as combination therapy.

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After learning of the MTOPS study from a friend, Richard contacted one of the trial sites directly. Richard participated in the 3-year trial as well as a 2-year follow-up study, and said, "I was very pleased to be a part of it."

Today, as a result of participating in a study... sponsored by the NIDDK, Richard said that the drug regimen he was put on has his BPH under control. "I'd definitely recommend others take part in such studies," said Richard. "It has improved the quality of my life, and I feel I'm in a much better position to make informed decisions about how to treat BPH."

As part of the study protocol, he was given a digital rectal exam to determine whether he actually had BPH, as well as a prostate-specific antigen (PSA) blood test and rectal ultrasound to rule out cancer. Participants were randomized to one of four treatment groups: double-placebo; finasteride alone; doxazosin alone; or the combination of the two drugs. Because it was a "double-blind" study, neither Richard nor his doctor was told of his assigned group until the study was over.

"I was required to take my pills every night and to have a digital rectal exam once a year," he said. He appreciated the fact that, while taking part in the study, the drugs were provided free of charge.

At its conclusion, the MTOPS study demonstrated that, compared to placebo, finasteride and doxazosin, in combination, reduced the risk of BPH progression 67 percent. In contrast, the risk of progression was reduced by 39 percent with doxazosin alone and by 34 percent with finasteride alone.

"Combination therapy not only provides better long-lasting [BPH] symptom relief," said MTOPS trial leader John McConnell, M.D., professor of urology and executive vice president of the University of Texas Medical Center in Dallas, "but because finasteride reduces prostate size, patients have fewer episodes of urinary retention and invasive treatments," including surgery. He added that the study also clearly demonstrated which patients are at increased risk of progression and thus most likely to benefit from treatment.

Richard continues to see his urologist once a year, has an annual digital rectal exam, takes his medications as prescribed, and instead of having to rouse himself from bed five to eight times to urinate, as he once did, he now averages only once a night. He reports experiencing no sexual dysfunction or other side effects, and said, "I couldn't be happier."

What is BPH?

The cause of BPH is not yet well understood. What is known is that it is common for the prostate to become enlarged as a man ages. The prostate is a walnut-sized gland that forms part of the male reproductive system. It is located in front of the rectum just below the bladder, where urine is stored. One of its main roles is to squeeze fluid into the urethra as sperm moves through during sexual climax. As the prostate enlarges, however, it may affect the function of the bladder. Consequently, typical BPH symptoms include:

- A slow, interrupted, weak urine stream;
- Urgency of the need to urinate;
- Leaking or dribbling of urine; and
- More frequent urination, especially at night.

Because the prostate gland plays a role in both sex and urination, many people feel uncomfortable talking about BPH. Nonetheless, more than half of men in their 60s and as many as 90 percent in their 70s have some symptoms of BPH. BPH rarely causes symptoms before age 40. Richard was in his late 40s when he first started to manifest symptoms of the condition, and he was wise to seek help.

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Studies show that in 8 out of 10 cases, the symptoms mentioned previously are indicative of BPH, which is common and treatable. However, these symptoms also can signal other, more serious conditions that require prompt treatment, including prostate cancer. Richard said he knows several men around his age whose symptoms were similar to his, but, after seeing their physicians, they learned they had prostate cancer.

"I'm grateful that my BPH was treated early; this is a problem that men should take seriously," said Richard.

Not to be discounted, severe BPH itself can cause serious problems over time. Urine retention and strain on the bladder can lead to urinary tract infections, bladder or kidney damage, bladder stones, and incontinence—the inability to control urination. If the bladder is permanently damaged, treatment for BPH may be ineffective. However, when BPH is detected in its early and moderate stages, there is a lower risk of developing such complications.

"I'm grateful that my BPH was treated early; this is a problem that men should take seriously," said Richard.

Improving Health Through Medical Research

Richard's improved quality of life results from the Nation's investment in biomedical research. There is much more that needs to be learned about BPH. To address this, the NIDDK continues to support research including studies to elucidate factors which contribute to onset, natural history, and alternative therapies.

In addition to clinical research, the NIDDK's Division of Kidney, Urologic, and Hematologic Diseases supports a robust Basic Cell Biology Program that supports a diverse array of projects with a primary emphasis on basic research in the bladder, prostate, urinary tract, kidney, and the lower reproductive system. This Program promotes scientific investigations aimed at understanding the fundamental cellular and molecular mechanisms operating under normal and diseased states. Supported work includes studies in human cells as well as in animal model organisms. Additionally, the Developmental Biology of the Kidney and Urogenital Tract Program encompasses studies that focus on fundamental cellular biology of the kidney and urogenital tract (bladder and prostate) and on mechanisms through which they develop.

PATIENT PROFILE

Diagnosis of BPH

Diagnosis of BPH usually occurs when a patient reports symptoms to his physician or when the physician, during a routine check-up, finds that the patient's prostate is enlarging.

Several tests help the doctor identify the problem and decide the appropriate form of treatment, including:

Digital Rectal Exam (DRE)—the physician inserts a gloved finger into the rectum to help determine the size and condition of the prostate.

Prostate-Specific Antigen, or PSA, Blood Test—helps to rule out cancer as a cause of urinary symptoms. Note: Much remains unknown about the interpretation of PSA levels, the test's ability to discriminate cancer from benign conditions of the prostate, and the best course of action following a finding of elevated PSA.

Rectal Ultrasound and Prostate Biopsy—a procedure whereby a probe inserted in the rectum directs sound waves at the prostate to help determine whether an abnormal-looking area is indeed a tumor. The doctor can use the probe and the ultrasound images to guide a biopsy needle to the suspected tumor to collect tissue specimens for further study under a microscope.

Urine Flow Study—the physician asks the patient to urinate into a special device that measures how quickly urine is flowing. A reduced flow often suggests BPH.

Cystoscopy—a small tube called a cystoscope, containing a light and a lens is inserted through the opening of the urethra in the penis to help determine the size of the gland and identify the location and degree of the obstruction.

Very seldom are all of the above tests necessary to make a diagnosis.

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