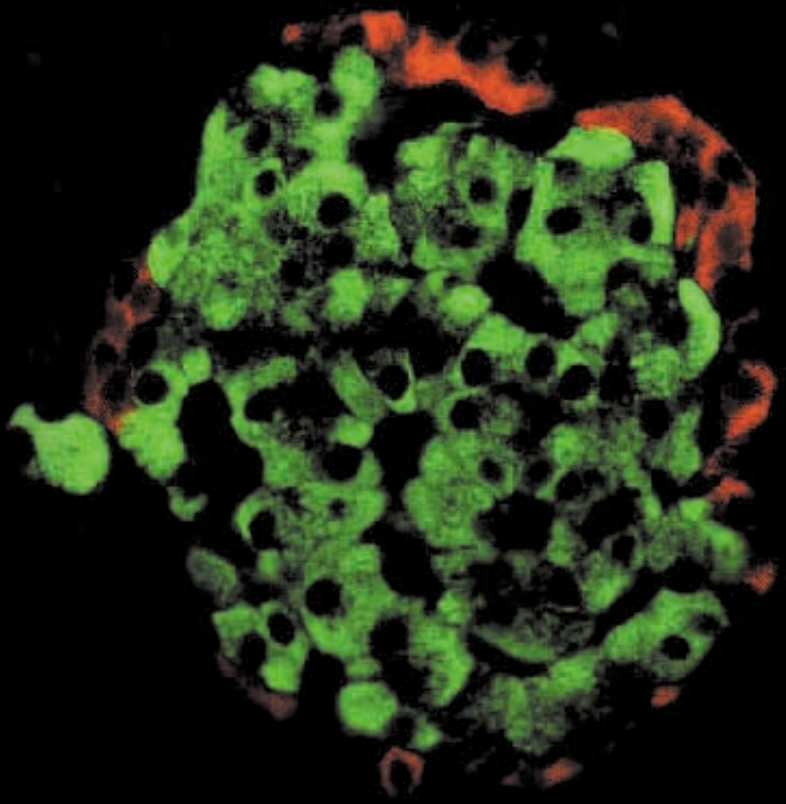

Diabetes, Endocrinology and Metabolic Diseases

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one-third of whom do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a role. Although there are several interventions currently available to help reduce the burden of this disease, there are no methods to cure or prevent it. The disease is marked by deficiencies in the body's ability to produce and properly use insulin—a hormone that is essential for the conversion of food-derived glucose into energy necessary for daily life. As a result, glucose becomes elevated in the blood, with detrimental effects on both small and large blood vessels. The most common forms of the disease are type 1 diabetes, in which insulin-producing capacity is totally destroyed, and type 2 diabetes, in which the body is resistant to insulin, even though some amount of insulin may be produced. Both forms of diabetes can lead to serious and costly complications, including kidney failure, blindness, amputations, heart disease and stroke. According to the American Diabetes Association, diabetes and its complications cost nearly $100 billion annually.

Type 1 diabetes most often occurs in children, but can appear at any age. Formerly known as insulin-dependent or juvenile-onset diabetes, it accounts for 5 to 10 percent of all diabetes in the U.S. It occurs equally among males and females, but is more common in Caucasians than in non-Caucasians. Type 1 diabetes develops when the body's system for fighting infection—the immune system—turns against itself in a disease process termed "autoimmunity." The immune system destroys clusters of cells in the pancreas called islets, which contain the body's insulin-producing beta cells. Once these cells are destroyed, type 1 diabetes patients require either lifelong insulin injections, often multiple times throughout the day, or infusion of insulin via a pump to control their blood glucose levels. Insulin therapy, however, is not a cure, nor can it always prevent the long-term complications of the disease.

Type 2 diabetes is the most common form of the disease. Once known as non-insulin-dependent or adult-onset diabetes, it affects about 90 to 95 percent of people with diabetes. Type 2 diabetes is more common in older people, especially older women who are overweight. Obesity is a major risk factor for this form of diabetes (see advances in obesity research on page 55). It also occurs more frequently among minority groups, including African Americans, Hispanic Americans, Native Americans, and Native Hawaiians. Recently, largely because of an increased incidence of childhood obesity, a disturbing increase of type 2 diabetes has occurred in children, particularly minority children. In patients with type 2 diabetes, cells in muscle, fat and liver tissue do not respond effectively to insulin. Gradually, the pancreas secretes less and less insulin in response to meals, and the timing of insulin secretion becomes abnormal. As clinically recognizable diabetes develops, production of insulin continues to decline. To control glucose levels, treatment approaches include diet, exercise and medications; some patients also need to take insulin.

Understanding and Combating the Disease Processes in Type 1 and Type 2 Diabetes

How the Insulin-Secreting Beta Cell Works

Gaining knowledge about the beta cells of the pancreatic islets is important to both type 1 and type 2 diabetes, because they are the key to insulin production

This photograph shows a pancreatic islet. It is comprised of many cells which produce the hormones that regulate blood glucose levels. These include insulin-producing cells (green) and glucagon-producing cells (red). Individuals with type 1 diabetes lack functional pancreatic islets and are therefore unable to produce insulin to regulate their blood glucose levels. Transplantation of islets holds the promise of a cure for type 1 diabetes and may free patients from a lifetime of multiple daily insulin injections and the disabling complications resulting from the disease. Photo: Dr. Gang Xu and Dr. Susan Bonner-Weir, Joslin Diabetes Center.

and resulting glucose control. Beta cells must "sense" the level of glucose in the blood and secrete the appropriate amount of insulin when glucose levels rise. In type 2 diabetes, researchers are studying the regulatory mechanisms by which the number of beta cells increases in response to the body's need for insulin, and also decreases by a feedback mechanism of programmed cell death, called "glucose-induced apoptosis." Type 2 diabetes occurs when beta cell death exceeds increases in beta cell capacity. In animal models susceptible to the development of type 2 diabetes, this imbalance appears to result from elevated blood glucose. In the laboratory, several groups of NIDDK-supported researchers have provided evidence for a link between the pathway of glucose metabolism, which leads to protein modification in the beta cell, and glucose-induced apoptosis. These findings are the first demonstration of a potential mechanism underlying the effect of severe or prolonged elevation of blood glucose in producing beta cell death and subsequent type 2 diabetes in genetically susceptible animals. More importantly, this research has identified a potential target for prevention of type 2 diabetes.

Mysteries of the beta cell can also be unlocked through the study of cultured cells obtained from animals. One such example, the INS-1 cell line, has been cultured from the pancreatic beta cells of rats. This cell line is an important tool for research studies because it is not subject to the inherent difficulties of obtaining fully-functioning islets from humans or animals. The INS-1 cell line provides a practical model for studying the biochemical mechanisms of insulin secretion in response to glucose. However, even this cell line is imperfect. The INS-1 cells exhibit a less vigorous secretion of insulin in response to glucose than do freshly isolated islets. In addition, the INS-1 cells lose their ability to respond to glucose during extended culture periods. To address these problems, NIDDK-supported researchers have inserted the gene for human insulin into the INS-1 cells in order to create new cell lines with a markedly improved ability to respond to glucose stimulation over long periods of time. Cells from this genetically engineered INS-1 cell line may prove valuable for further defining the mechanisms involved in the regulation of insulin secretion, which may possibly lead to the development of new therapeutic agents for treating type 2 diabetes. This research is also highly relevant to type 1 diabetes because cell lines that more faithfully mimic the function of normal pancreatic islets could possibly replace islets that have been destroyed by the autoimmune process in that disease.

Beta cell function is also being discerned more precisely through the application of another research tool—animal models of diabetes. Such models provide an essential resource for understanding the disease and its complications in humans, and for testing potential interventions. Recently, several independent groups of investigators have produced genetically altered mice, which will likely shed new light on diabetes. They have generated "knockout" mice, in which specific genes are deleted, and "transgenic" mice, which carry inserted genes targeted to specific cells. These animal models have increased understanding of important genes involved in the regulation of beta cell growth and insulin secretion—knowledge that is essential to understanding and counteracting the disease processes in both type 1 and type 2 diabetes.

The NIDDK is capitalizing on the enormous opportunities for discoveries in beta cell research by establishing a Beta Cell Biology Consortium—a major recommendation of the congressionally established Diabetes Research Working Group. By using a consortium approach, the NIDDK will provide scientists with access to information, resources, technologies, expertise, and reagents that are beyond the means of any single research effort. A comprehensive understanding of the molecular basis of beta cell development and function will help to generate new research tools and provide critical insights into the prevention and treatment of both type 1 and type 2 diabetes.

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Insulin Action

To comprehend diabetes fully, it is critical to identify the mechanisms by which insulin exerts its action on cells of the body. This knowledge is especially important for type 2 diabetes patients, many of whom continue to produce insulin, although at inadequate levels. However, their bodies are resistant to the effects of insulin. In order to respond properly to insulin's signal, a cell must have insulin receptor (IR) proteins on its surface to which insulin can bind and thus exert its metabolic effects. This sensing and binding process triggers within the cell a cascade of events, which results in the uptake of glucose from the blood and its use as energy. In order to characterize the proteins that relay the insulin signal within the cell, scientists have studied genetically engineered mice in which the deletion or "knockout" of certain genes causes the absence of proteins important in the insulin signaling pathway. The missing proteins were the IR itself, and two other proteins that mediate insulin action, IRS-1 and IRS-2. By using "knockout" mice as a model of diabetes, researchers have demonstrated that IRS-1 plays a major role in stimulating glucose uptake by muscle and fat, while IRS-2 is more important in the liver. Studies such as these have demonstrated that the liver plays a key role in the development of type 2 diabetes and that insulin resistance must occur in both muscle and liver for development of the disease. Interestingly, these same metabolic pathways may also affect fat metabolism, which may explain the association of type 2 diabetes with obesity. For example, researchers have demonstrated that the insulin receptor in the brain plays an important role in the regulation of food intake, which may be a critical factor in obesity and its strong association with type 2 diabetes.

In further efforts to define how insulin signaling and transport occur, researchers have identified another pathway involved in insulin-stimulated uptake of glucose by fat and muscle cells. A protein, CAP, has been shown to play a major role in this signaling pathway. High levels of CAP are found in muscle and fat, and the presence of CAP increases in fat cells in response to medications that make cells more sensitive to the effects of insulin. Future research will lead to increased understanding of this particular signaling pathway and may uncover additional mechanisms essential for insulin's metabolic effects.

These and other research accomplishments have helped to elucidate some of the important steps in insulin action. Such basic research progress provides the impetus for critically needed targets for new drug development and other potential treatments for diabetes.

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Abnormal Immune Function in Type 1 Diabetes

For unknown reasons, individuals with type 1 diabetes often develop antibodies against proteins in the beta cells, including insulin itself—leading to autoimmune destruction of insulin-producing capacity. Based on this knowledge, researchers have used antibodies to discover methods for predicting, with great accuracy, whether certain relatives of type 1 diabetes patients are at high-risk for developing the disease. Now, a recent study has found that high levels of antibodies to insulin in infants and young children correlate with later development of type 1 diabetes. This finding may help to identify at-risk individuals and thus provide an opportunity to apply prevention strategies, before insulin-producing capacity is completely destroyed.

In order to understand exactly how the cells of the immune system distinguish between normal and poten-

tially harmful proteins, researchers have examined the Major Histocompatibility Complex (MHC), which is known to play a key role in activation of the body's immune defense system. Found on the surface of cells of the immune system, the MHC helps identify potentially harmful antigens. Scientists have discovered that mice susceptible to diabetes have an abnormally shaped MHC, which permits it to interact with a wider range of antigens. As a result, normally harmless proteins could be erroneously identified by the animal's defense system as a "threat" that might trigger an inappropriate and harmful immune response. Some people with type 1 diabetes have an MHC (called HLA, for human leucocyte antigen, in humans), whose structure parallels that of diabetic mice, suggesting that a similar mechanism may lead to development of the autoimmune disease process that underlies type 1 diabetes in both mice and humans.

In related work on the MHC, researchers have also found that, in mice normally resistant to diabetes, replacement of mouse MHC with a particular human HLA known as DQ8 will cause the mice to develop diabetes spontaneously. This form of HLA has previously been shown to increase susceptibility to diabetes in humans. Conversely, the HLA called DQ6, which is known to confer protection from diabetes in humans, can prevent the development of the disease in mice. This research provides insights into which components of the immune system are critical in promoting susceptibility to or protection against the development of type 1 diabetes. Models such as these may offer a precise system to examine disease progression and to test specific therapeutic interventions in patients.

The autoimmune response underlying type 1 diabetes may be triggered by environmental factors. Infection with coxsackievirus, a common enterovirus, may be one environmental factor that could contribute to type 1 diabetes in genetically susceptible individuals. Using a mouse model of diabetes, researchers have found that infection with a coxsackievirus affecting the pancreas can accelerate type 1 diabetes development once beta cell destruction has begun. However, coxsackievirus infections do not appear to initiate beta cell destruction. These findings indicate that the timing of an infection may have important implications for the development of the autoimmunity seen in type 1 diabetes. This new understanding of the role of the environment in triggering the immune system may lead to methods for modulating the immune response in a way that could mitigate or completely prevent beta cell destruction.

Knowledge of the immune response gained from previous studies made it possible for the NIDDK to undertake a major clinical trial, the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1). The scientific rationale for initiating the DPT-1 was based on earlier research results suggesting that immune tolerance provoked by presentation of oral insulin to the immune system *via* the intestinal mucosa, or injected insulin, could reduce pancreatic islet autoimmunity, leading to a delay in the onset of type 1 diabetes. The major objective of the ongoing DPT-1 is to determine whether early intervention, using the antigen-based therapies of insulin injections or oral insulin, can delay the onset of the disease in at-risk, non-diabetic relatives of individuals with type 1 diabetes. The DPT-1 is jointly supported by the NIDDK, the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, the National Center for Research Resources, the American Diabetes Association, the Juvenile Diabetes Research Foundation International, and industry.

A new NIDDK initiative that will greatly facilitate additional clinical research on type 1 diabetes is the Type 1 Diabetes TrialNet–an approach recommended by the congressionally established Diabetes Research Working Group. Spearheaded by the NIDDK, this effort is cosponsored by the National Institute of Allergy and Infectious Diseases and the National Institute of Child Health and Human Development. The TrialNet will include clinical centers, recruitment networks and a coordinating center. It will provide the research infrastructure needed to spur the future design and execution of pilot studies and expanded clinical research. With the TrialNet, more rapid clinical testing of novel approaches to treatment and prevention will be possible, as soon as they emerge from fundamental investigations. The TrialNet will thus enable efficient performance of intervention studies to preserve pancreatic beta cell function in new-onset cases of type 1 diabetes, and ultimately, to prevent onset of the disease.

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Genetics of Diabetes and Its Complications

Diabetes and its complications have strong genetic determinants. Even though diabetes appears to develop as a result of many factors, virtually all forms appear to have genetic influences, with the likely involvement of multiple genes. Because several genes are involved, diabetes is termed a "complex" genetic disease, which is particularly challenging to understand.

In type 1 diabetes, the destructive autoimmune disease process is influenced by the genes that control the immune system. Research has shown that the strongest genes predisposing to type 1 diabetes are common genetic variations of the Major Histocompatibility Complex (MHC) molecules known as human leukocyte antigens (HLA). About 85 to 90 percent of individuals with type 1 diabetes are positive for the MHC molecules of the HLA types known as DR3 or DR4. In addition, people who have those HLA types, but do not yet have type 1 diabetes, are much more likely to develop it than are persons with other HLA types. In addition to the HLA genes, there are environmental influences, as well as other genes, that influence susceptibility to type 1 diabetes.

In type 2 diabetes, the development of the technology to perform genome-wide screens has led to the localization of disease susceptibility genes in a number of populations from the U.S. and Europe. Separately and in combination these studies have localized susceptibility regions to several different areas of the genome, reinforcing the polygenic nature of the disease. Because of this genetic complexity, researchers initially focused on rare forms of the disease—accounting for 5 percent of all cases—in the hope that they would provide clues about more common forms of the disease. For some time, scientists have known that single-gene mutations can cause rare forms of diabetes, such as Maturity Onset Diabetes of the Young (MODY) and other rare subtypes of type 2 diabetes. Five such genes have been identified which, when altered, can result in the development of MODY. However, these genes seem to play a relatively minor role in type 2 diabetes, the more common and more difficult form of the disease to comprehend genetically.

In the quest for disease susceptibility genes, one group of investigators recently described two mutations in the *NEUROD1* gene that are associated with the development of type 2 diabetes. The protein encoded by this gene functions as a regulatory switch for the development of that portion of the pancreas that secretes insulin and glucagon—hormones responsive to blood glucose levels. In a mouse model with two disrupted copies of this gene, pancreatic islet development is abnormal and overt diabetes develops. In order to determine if *NEUROD1* plays a causative role in type 2 diabetes in humans, investigators screened over 90 individuals with the disease for mutations in this gene and identified two families with mutations. It is possible that the development of type 2 diabetes in carriers of either of these two mutations may result from a disruption of gene activity in the islets.

In another genetic study, NIDDK-supported investigators in collaboration with French researchers have demonstrated that mutations in the insulin-promoting factor (*IPF-1*) gene may be related to the development of type 2 diabetes. *IPF-1* is critical for embryonic development of the pancreas and for regulation of endocrine pancreas-specific genes in adults. Some individuals with mutations in *IPF-1* develop MODY. Three novel *IPF-1* mutations were found in over 60 unrelated individuals of French ancestry with type 2 diabetes. This is the first evidence that *IPF-1* may represent a diabetes-predisposing gene in a portion of individuals with the common form of type 2 diabetes.

The gene hunt in type 2 diabetes reached a major milestone in 1996 when investigators reported the localization of a disease susceptibility gene on chromosome 2 in a Mexican American population. They found that the candidate gene, which they called *NIDDM1*, interacts with another gene on chromosome 15 to increase diabetes susceptibility. Now, this finding has been extended by the recent discovery that a gene known as *calpain-10* shows an association with type 2 diabetes in Mexican Americans and in two Northern European populations. This gene

appears to be the gene on chromosome 2 that was first linked to susceptibility to type 2 diabetes and termed *NIDDM1*. Individuals in the study populations, who have a certain combination of genetic markers, also have approximately a three-fold increased risk of developing type 2 diabetes. The presence of the protein encoded by the *calpain-10* gene in insulin-producing pancreatic islets, muscle and liver suggests that it could affect insulin secretion, insulin action and glucose production by the liver—all functions altered in type 2 diabetes. These results point to new pathways that may be involved in the regulation of blood glucose and the development of diabetes, and may reveal new targets for treatment.

The *calpain-10* gene has also been studied in the Pima Indians of Arizona, who have the highest reported prevalence of diabetes in the world. Their diabetes is characterized by obesity, resistance to the action of insulin, changes in insulin secretion, and increased glucose production. In a recent study, certain Pima Indians were found to have reduced expression of the *calpain-10* gene in muscle tissue. These individuals also had insulin resistance, apparently due to lower rates of insulin-stimulated glucose utilization. These results provide the first evidence in humans for a functional biological change resulting from a mutation in the *calpain-10* gene.

Genetic clues about type 2 diabetes are also emerging from research on the lipodystrophies—a group of disorders characterized by selective loss of fat from various parts of the body. Some individuals with this condition may have only cosmetic problems, while others may suffer from metabolic complications in varying degrees of severity. The degree of fat loss determines the severity of metabolic complications related to insulin resistance, such as those seen in type 2 diabetes, and in elevated blood lipids. These disorders can be either inherited or can occur secondary to various types of illnesses or drugs. One of the inherited lipodystrophies, familial partial lipodystrophy (FPLD), is characterized by normal fat distribution at birth and during childhood. However, at the onset of puberty, individuals lose fat from the extremities, trunk and buttock regions of the body, while excess fat may appear in the face, neck and back. In women, lack of fat in the buttocks is striking and they may complain of "no hips" or "flat hips." Previously, researchers localized the gene for FPLD to a region of chromosome 1. Other investigators have linked mutations in the gene that directs the production of the protein lamin A/C, a component of the double-layered membrane enclosing the nucleus of the cell, with a form of muscular dystrophy, and, most recently with FPLD. Further research has demonstrated four independent mutations within a particular region of the *lamin A/C* gene in members of 14 families affected by FPLD. This is the first gene known to be altered in the lipodystrophies and may provide clues into complex metabolic problems, such as those seen in type 2 diabetes, by defining new pathways that regulate fat cell mass and insulin sensitivity.

Genes are a critical factor not only in the onset of type 1 and type 2 diabetes, but also in the onset and progression of the complications of diabetes. For example, previous studies have suggested a familial clustering of diabetic kidney disease. More recent investigations have demonstrated that one major gene, as yet unidentified, may increase susceptibility to the kidney disease of diabetes in the Pima Indian population of Arizona, who suffer tremendously from diabetes.

In related basic research, NIDDK grantees are seeking to create animal models to study genetic factors in diabetic complications. Technology has advanced to the point that it is theoretically possible to genetically engineer mice so that they develop diabetic complications similar to the major human complications resulting from diabetes. These animal models would be especially valuable for analyzing the initiation and progression of diabetic complications; for providing a framework for discovery of genes and cellular factors that result in susceptibility or resistance to complications; for providing targets for intervention and treatment; and for permitting tests of prevention, detection, therapeutic, and imaging strategies.

Recent advances in research thus provide a compelling opportunity to understand more fully the underlying genetic factors that may make certain individuals more susceptible to the onset of type 1 or type 2 diabetes, and then, to the initiation and severity of diabetic complications. This knowledge may lead to improved prevention and treatment strategies that could put a halt to this devastating disease. In order to capitalize on genetic discoveries in type 1 and type 2 diabetes research, the NIDDK is involved in major initiatives. The Institute is a leading partner in support of the International Type 2 Genetic Linkage Analysis Consortium. The purpose of the Consortium is to combine data from multiple genome scans and thus increase the probability of gene

discovery. The groups involved in the Consortium are pursuing a fine mapping effort of potential susceptibility genes for type 2 diabetes located on chromosome 20. Intensified support for the Consortium will also permit additional analyses to determine whether unique susceptibility genes exist in African Americans, who have a disproportionately heavy burden from type 2 diabetes. On a parallel track with efforts on the genetics of type 2 diabetes, the NIDDK and the Juvenile Diabetes Research Foundation International are working closely to establish a Type 1 Diabetes Genetics Consortium. The initial objective would be to pursue the results of three genome-wide scans for type 1 diabetes, which have recently been completed. These scans have identified several genetic regions as containing diabetes susceptibility genes. A combined analysis of these three datasets could identify the most promising avenues in the quest for genes that confer susceptibility to type 1 diabetes. With respect to the complications of diabetes, the NIDDK is also working across the Divisions of the Institute and in conjunction with other NIH components to identify the genes responsible for the kidney complications of diabetes. As part of this effort, the Institute is now launching a new genetics initiative called "FIND"—the Family Investigation of Nephropathy and Diabetes. This initiative will focus on family studies designed to uncover candidate genes associated with type 1 or type 2 diabetes, genes associated with development of complications, and genes relevant to those identified in animal models. A specific objective will be to search for susceptibility genes in subpopulations of Caucasians, African Americans, Hispanic Americans, and Native Americans across the U.S.

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Seeking a Cure for Type 1 Diabetes through Islet Transplantation

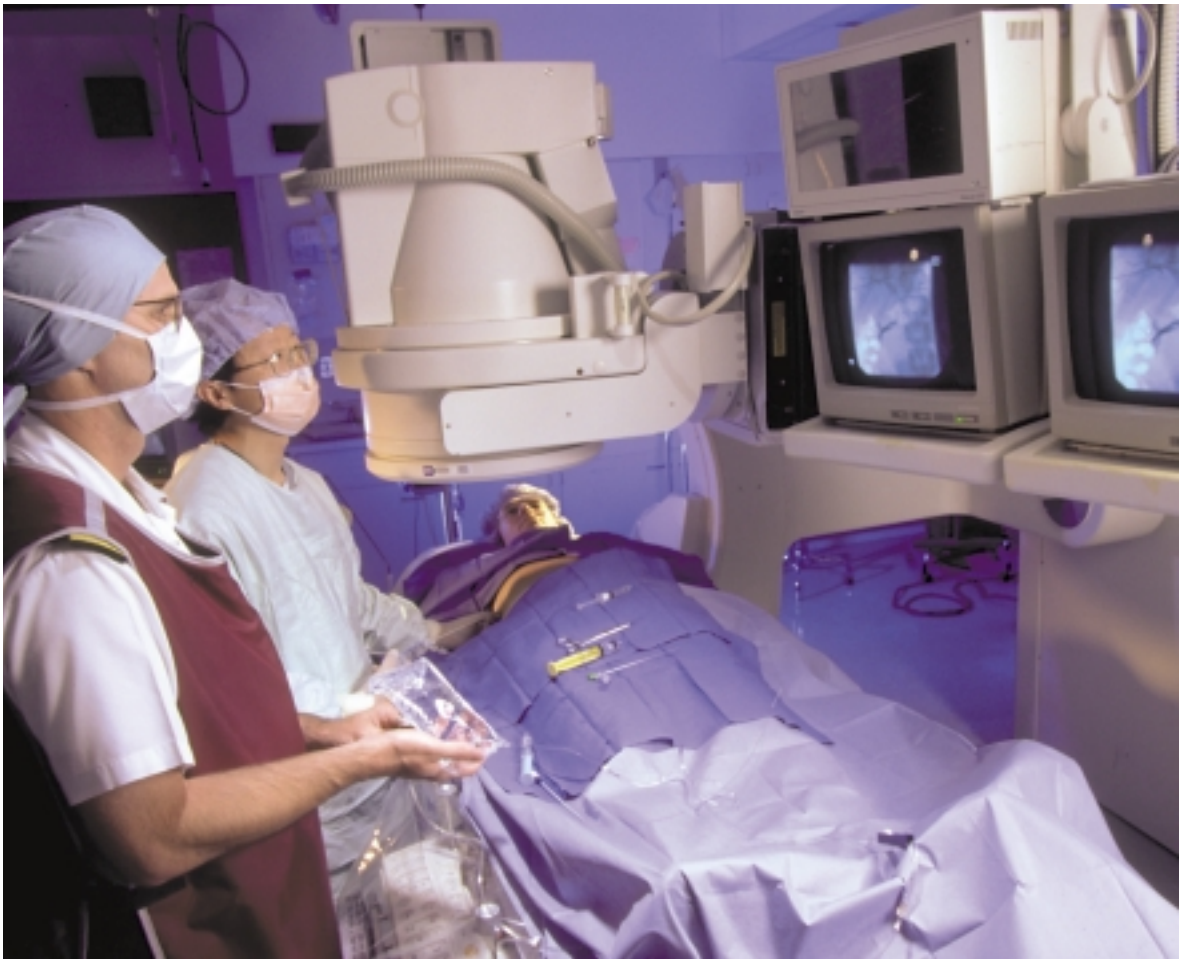
For decades, researchers have been searching for better ways to treat, prevent, and ultimately cure type 1 diabetes. They have pursued means other than the external administration of insulin to regulate blood glucose levels and/or to restore insulin-producing capacity. Successful transplantation of the whole pancreas is the most common procedure to re-establish normal blood glucose regulation; however, this procedure entails major surgery and is usually done only in conjunction with a kidney transplant in those individuals with end-stage renal disease. Moreover, it is not a feasible therapy for young children with type 1 diabetes. Therefore, scientists have been concentrating on a method for replacing only the insulin-producing islets isolated from donor pancreas. Until recently, success has been hampered by numerous factors. These include: (1) the requirement for lifelong immunosuppressive therapy to prevent rejection of the transplant, and (2) the difficulty of obtaining adequate numbers of islets for transplantation. Through research conducted and supported by NIDDK investigators, vital groundwork has been laid for overcoming these challenges.

Preventing Transplant Rejection: Immunosuppressive agents used to prevent transplant rejection can cause injury to the transplanted islets and severe side effects. Moreover, they are not effective in all patients. In searching for alternatives to these drugs, researchers have found that rejection of transplanted islets can be avoided if the immune system is "re-educated," so that it will accept new

islets as "self" and not see them as "foreign" tissue. This re-education requires "switching off" one function of critical cells active in the immune response called T-cells. These T-cells are involved in a two-stage activation process to protect the body from foreign elements that pose a danger, such as bacteria and viruses. One stage recognizes that the foreign entity is not a normal part of the body. The second stage, called "co-stimulation," leads to the production of other T-cells that attack and destroy the foreign material or organism. When researchers block co-stimulation, the immune system learns to accept the foreign entity as "self," a process called "tolerization" or "tolerance induction." Therapies based on tolerization raise the possibility of selectively modifying the immune system's reactions so that it will not reject transplanted tissue, thereby avoiding the need for suppressing the entire immune system indefinitely with anti-rejection drugs.

NIDDK-supported investigators have recently published data on successful islet transplantation in animals without the need for long-term immunosuppression. This approach resulted in improved metabolic function in non-human primates. They transplanted islets isolated from the pancreata of non-diabetic monkeys into the portal vein of diabetic monkeys. The animals then received an immunotoxin for four days following surgery. Subsequent metabolic testing of the animals demonstrated that control of blood glucose had been achieved: none of the animals required insulin injections nine days post-transplantation. Tolerance induction alleviated the need for long-term immunosuppression. After this short-term immunotoxin treatment, the monkeys did not experience any apparent immunological compromise, such as infections or malignancies.

In clinical studies, the refinement of islet transplant techniques has culminated in a remarkable achievement. A new technique, the "Edmonton Protocol"—developed by researchers at the University of Alberta, Canada—garnered international headlines after the announcement that a number of type 1 diabetes patients experimentally treated with islet transplants had remained insulin-free for up to 14 months. This protocol uses a novel, steroid-free combination of three drugs, which not only prevents rejection, but also halts further autoimmune destruction



Left: Dr. Allan Kirk, a transplant surgeon in the NIDDK's new Transplantation and Autoimmunity Branch, discusses a clinical research protocol with a patient. *Right:* Surgeons perform experimental islet transplants with the aid of sophisticated imaging devices, which enable them to implant the islets with great precision. Photos: Mr. Richard Nowitz.

of the islets. In this technique, islets are isolated from the pancreas of organ donors. Following isolation, the islets are injected into the portal vein, which supplies blood to the liver. The islets then migrate to the liver, where they flourish and produce sufficient insulin and almost perfect control of blood glucose. This protocol is now being tested in a larger number of patients.

Ensuring a Plentiful Supply of Islets for Transplantation: If the promise of islet transplantation is realized in studies of larger numbers of patients, a major obstacle to its widespread therapeutic use will be limitations in tissue supply. Only a few thousand human pancreata are currently available from organ donors for islet isolation in the U.S. each year. This number is inadequate to provide islet transplant therapy to the approximately 800,000 individuals with type 1 diabetes. To address this shortfall of islets, the NIDDK is pursuing research along several fronts.

One answer to the tissue supply problem may arise from advances in research on undifferentiated progenitor cells, called stem cells. Such cells might be "coaxed" into becoming insulin-producing cells for transplantation therapy. In laboratory work, NIDDK-supported investigators have generated islets using animal cells that are "pluripotent," meaning that they can give rise to many types of specialized cells. The researchers isolated cells from the pancreatic ducts of non-obese, diabetes-prone mice, prior to the onset of diabetes. These pancreatic duct cells produced islets capable of responding to glucose throughout a long-term culture period. When the islets were implanted into the kidneys of non-obese mice with overt diabetes, the mice were able to be weaned off insulin injections and exhibited a reduction in blood glucose levels, which approached normal levels. Future experiments will be necessary to verify that the observed reduction in glucose levels was the result of the implants. Additional research should increase understanding of the environment within the mouse that allows the laboratory-generated islets to further mature and differentiate into fully-functioning islets with maximal insulin-producing capacity. Although the focus of this research has been on the insulin-producing beta cells in the pancreas, hormones produced by other endocrine cells of the pancreas are also important in glucose regulation. Thus, the labo-

ratory-generated islets may represent an important research finding that could be applicable, not only to diabetes, but also to other diseases.

The objective of increasing the supply of islets may also be realized from studies of NIDDK-supported investigators on pancreatic duct cells—cells which would normally be discarded in the process of preparing human islets for transplant. Researchers have shown that these cells can be encouraged to form insulin-producing cells called cultivated human islet buds (CHIBS). During the culturing and expansion of the CHIBS, insulin content was increased ten-to-fifteen-fold and the tissue became more organized into islet-like structures. The islet buds were able to secrete insulin in response to glucose stimulation—the same form of sugar that normally induces insulin release in the body. Although the number of islets generated is too small to be useful in a clinical setting, these findings certainly raise the possibility that, with further optimization, this technique might have major implications for use in islet replacement therapy in the future.

Augmentation of the supply of islets may also derive from the development of an automated method for isolating large numbers of islets from a single pancreas, thus making it possible for one pancreas to provide sufficient islets for transplantation. Until recently, as many as five or six organs were needed to yield the cells necessary for a transplant for a single patient; the "Edmonton Protocol" requires two organs for each islet transplant. Despite advances in islet harvesting techniques, however, the potential demand for islets still exceeds the available supply of organs. Therefore, scientists are exploring ways to develop alternative sources of islets by bioengineering human cell lines, by replicating beta cells from humans, or by manipulating other types of cells to become beta cells.

The congressionally established Diabetes Research Working Group set forth islet transplantation as an extraordinary research opportunity. In pursuing this area of research, the NIDDK, in a collaborative effort with the National Institute of Allergy and Infectious Diseases (NIAID) and the Juvenile Diabetes Research Foundation International, has funded numerous centers to develop improved protocols for islet transplantation in humans. In addition, the NIDDK Division of Intramural Research has initiated a clinical research program that will explore new approaches to both kidney and islet transplantation for diabetes—in collaboration with the Department of Defense, the NIH Clinical Center, and the Diabetes Research Institute of the University of Miami. The NIDDK and the Juvenile Diabetes Research Foundation International are also cosponsoring the Immune Tolerance Network (ITN) initiative, a collaboration involving numerous research institutions spearheaded by the NIAID. The ITN will solicit, develop, implement, and address clinical strategies, including biological assays, for the purpose of inducing and maintaining immune tolerance in patients receiving kidney and islet transplants. The Organ/Tissue Transplant Research Center of the NIDDK Division of Intramural Research is one of 11 centers participating in the ITN's testing of the "Edmonton Protocol" in performing transplant procedures in a larger number of patients. This clinical research will further assess the effectiveness of the technique and identify any long-term risks associated with steroid-free immunosuppressive therapies. Researchers hope the study will serve as a platform for testing new treatments in which the permanent reversal of diabetes can be achieved without the lifelong need for immunosuppressive drugs. In addition, the NIDDK will support two additional studies to determine if one pancreas can provide sufficient islets for a transplantation. In support of all of these efforts, the National Center for Research Resources of the NIH is establishing several centers aimed at maximizing islet-harvesting processes. As these multiple efforts converge, type 1 diabetes research is on a critical threshold of progress. With the removal of technical barriers through research, a "cure" for type 1 diabetes could indeed move from being a fervent hope and goal toward becoming a reality for all patients.

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Improving Management of Type 1 Diabetes through Glucose Sensors

While a major research priority is to find a cure for type 1 diabetes, it is also vital to improve existing methods of glucose control for individuals who already have the disease and desperately want to prevent or delay the onset of complications. Although major clinical trials have shown that close control of blood glucose levels can significantly prevent or delay complications, the optimal technology to achieve these results does not yet exist. Currently, excellent control requires careful glucose monitoring and either multiple daily injections of insulin or use of an insulin pump—an extremely difficult regimen to follow, especially for children and teenagers. In addition, a risk of precise blood glucose control is the development of dangerously low blood sugar, which could cause loss of consciousness, seizures, or other problems.

One of the greatest improvements in the management of both type 1 and type 2 diabetes in the past two decades has been the introduction and widespread implementation of reliable, accurate, and "user-friendly" self-glucose-monitoring devices. One promising approach thus far is an implanted insulin delivery system controlled by the patient, called an "open loop" system. Ideally, future research will uncover ways to make this system totally independent of the need for manipulation by the patient. Such a mechanical system, called a "closed loop" system, would combine automatic glucose sensing with automatic insulin delivery.

Progress has been made in the development of enzymatic glucose sensors in several forms: intravenous implants; needle-like probes penetrating the skin with the sensor tip in the tissues just below the skin; or devices fully implanted underneath the skin. Researchers recently implanted two such devices in an animal model of diabetes, with promising results. Although similar in design, one of the sensors was constructed to encourage the formation of capillaries adjacent to the sensor to better ascertain blood glucose levels. Glucose measurements obtained by the sensor were sent by radio waves to a receiver coupled with a computer. Stable, clinically useful performance of these sensors was demonstrated as early as seven days after implantation, and for a lifetime of three to five months. Investigators feel that this technology can be further optimized by using microcircuitry, which would miniaturize the sensor to approximately the size of a large jelly bean, implantable in an outpatient setting with local anesthesia.

In contrast to implantable enzymatic sensors, a newer, non-invasive method is currently under development—optical sensors. It is based on the principle that the absorption of near-infrared light can be quantitatively related to glucose concentration. Researchers have recently described progress in the development of a sensor that uses beams of light, passed through the fat pad of the thumb, which are then scattered by a mirror-like device. The resulting spectrum of light is collected and analyzed by a computer. Subsequent glucose measurements correlated with and accurately predicted blood glucose levels in the low and normal range in humans. Such non-invasive approaches are appealing because they may make it easier for individuals, especially children, to self-monitor blood glucose levels more frequently. While improvements continue in optical technology, these devices are still experimental. More research is needed on their reliability in measuring the wide range of glucose levels observed in diabetes, across the diverse spectrum of diabetes patients.

These and other exciting advances in continuous glucose monitoring lead to hope that an implantable artificial pancreas may one day become a reality. An artificial pancreas would likely have several facets: an insulin pump implanted under the skin; a blood glucose sensor with an external readout such as a wristwatch; and a control computer located within the pump that can be programmed externally. All of these components could function interactively by communicating with each other through the use of radio waves. The development of a clinically-useful artificial pancreas could aid in preventing or delaying the devastating complications of diabetes, while freeing patients from the discomfort of thousands of finger sticks and insulin injections.

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Caroline Rowley – Type 1 Diabetes

Caroline Rowley was six years old when her grandmother, who hadn't seen Caroline for nine months, came to visit. "Caroline looks thin and frail, and seems very cranky," her grandmother noted. Caroline's mother replied that she was probably just losing some of her baby fat. But others noticed a difference in the strawberry blond, blue-eyed little girl, as well. "She seems tired," said the mother of one of Caroline's friends. And the school nurse reported that Caroline had been in the nurse's office every day for two weeks complaining of a stomachache. Meanwhile, Caroline was urinating frequently, was constantly thirsty and hungry, and her vision had become blurry. Her mother thought Caroline had a bladder infection or perhaps mononucleosis. Finally, Caroline was diagnosed with type 1 diabetes. "The diagnosis changed our lives," says Caroline's mother, who adds that there is no history of diabetes in the family. "Imagine telling your six-year-old that she's going to have to prick her finger and take shots at least four times every day for the rest of her life to stay alive. It was awful and had an impact on our entire family."

Caroline Rowley was diagnosed with type 1 diabetes at age six. The diagnosis affected the entire family, says her mother, Carol (right), who likens the maintenance of Caroline's blood glucose levels to a "daily ongoing chemistry experiment." New research into type 1 diabetes, including the transplant of islets, offers hope for the future. Now 10 years old, Caroline says, "I don't think I'll let diabetes affect my hopes and dreams."

Today, four years later, Caroline's family lives in constant fear of the serious damage diabetes may inflict on their 10-year-old daughter and in hope that a cure can be found soon for this devastating autoimmune disease that affects virtually every organ system of the body.

The good news is that research conducted and supported by the NIDDK, as well as other organizations, may soon turn the hope of the Rowley family—and the families of the estimated 16 million other Americans who suffer from diabetes—into reality.

About Diabetes

Diabetes affects the way our bodies use digested food for growth and energy. Most of the food we eat is broken down by digestive juices into a simple sugar called glucose. After digestion, the glucose passes into our bloodstream, where it is available for body cells to use for growth and energy. For the glucose to get into the cells to do its work, the presence of insulin—a hormone produced by the pancreas—is required.

When we eat, the pancreas is supposed to automatically produce the right amount of insulin to move the glucose from our blood into our cells. In people with type 1 diabetes, however, the immune system attacks the insulin-producing beta cells in the pancreas and destroys them. As a result, glucose builds up in the blood, over-

flows into the urine, and passes out of the body, depriving the body of its main source of fuel, even though the blood contains large amounts of glucose.

Individuals with type 1 diabetes need daily injections of insulin to regulate their blood sugar levels—and to live. Insulin is by no means a cure for diabetes, but before its discovery in 1921, everyone with type 1 diabetes died shortly after the onset of the disease. At present, scientists do not know exactly what causes the body's immune system to attack the beta cells, but they believe that both genetic and environmental factors are involved.

Currently, there is no cure for type 1 diabetes. However, an important NIDDK-supported nationwide clinical trial, called the "Diabetes Prevention Trial for Type 1 Diabetes" (DPT-1), is seeking to determine if type 1 diabetes can be prevented in individuals at immunologic, genetic, or metabolic risk for the disease by administering small, regular doses of insulin. Nine medical centers and more than 350 clinics in the U.S. and Canada are taking part in the trial.

Living with Type 1 Diabetes

People with diabetes fight a constant battle to keep their blood glucose levels from going too low or too high. The importance of closely maintaining blood glucose levels was revealed in a 10-year NIDDK-supported clinical trial, called the "Diabetes Control and Complications Trial." The study demonstrated that keeping blood sugar levels as close to normal as possible significantly slows the onset and progression of the eye, kidney, and nerve diseases caused by diabetes. For the Rowley family, however, maintaining Caroline's glucose levels has been a nightmare. "It's a daily ongoing chemistry experiment," says her mother.

Advances in diabetes research, however, are leading to better ways to manage the disease and more effective therapies to treat its complications. For example, through research:

- New forms of genetically engineered insulin are enabling many, but not yet all, people with diabetes to lead more normal lives.
- Better ways are being found to monitor blood glucose levels at home.
- Certain medications, called ACE inhibitors, can prevent or delay kidney failure in people with diabetes.
- External and implantable insulin pumps can deliver appropriate amounts of insulin and thus replace the need for daily injections in some patients.

"Before Caroline began using an external insulin pump we lived by the clock," her mother says. "She would sometimes sit on the floor and cry in frustration because she was either hungry, but couldn't eat, or wasn't hungry, but needed to eat to maintain a healthy blood sugar." Insulin pumps are not yet a true "pancreas-in-a-pocket;" they still need to be manually adjusted for various glucose and physical activity levels. But for many people they are better than multiple injections of insulin on a daily basis. It is expected that in the near future, as a result of continued research, advanced glucose sensing devices that can be implanted in the body will be available to Caroline and the approximately one million others with type 1 diabetes. These pumps would be able to respond automatically by sensing when insulin is needed and infusing the correct quantities exactly at that time.

TYPE 1 DIABETES FACTS

- **Type 1 diabetes is an autoimmune disease that affects the way our bodies use digested food for growth and energy.**
- **The disease gradually destroys the pancreatic insulin-secreting cells (beta cells), which leads to life-long dependence on insulin for survival.**
- **Although the disease develops most often in children and young adults, it can appear at any age.**
- **Symptoms include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue.**
- **If not diagnosed and treated with insulin, a person can lapse into a life-threatening coma.**

Promising New Research

Transplantation: Currently, a combined pancreas/kidney transplant offers the best hope of a "cure" for people with type 1 diabetes. Although these transplants generally are successful, this is a major surgical procedure. Moreover, recipients must take powerful drugs for the rest of their lives to suppress the immune system in order to prevent rejection of the transplanted organs. Such drugs have serious side effects. Only patients with kidney failure are considered for and receive such transplants. Moreover, because the anti-rejection drugs are so toxic, only under very unusual circumstances would a child undergo a transplant.

One of the most promising avenues of clinical research—supported by the NIDDK, the National Institute of Allergy and Infectious Diseases, and the Juvenile Diabetes Research Foundation International—is the transplantation of clusters of insulin-producing cells, known as islets. New methods are also being investigated to prevent rejection of the transplanted cells by inducing the immune system to tolerate them as if they were the recipient's own tissue. This approach may one day enable patients to remain free of insulin injections and eliminate the need for immunosuppressive drugs. Researchers already have developed an automated method for isolating large numbers of islets from a single pancreas. However, the demand for islets still exceeds the available supply of organs. Therefore, scientists are currently exploring ways to develop alternative sources of islets by bioengineering human cell lines, obtaining islets from other species, replicating beta cells from humans and other species, or manipulating other kinds of unspecialized cells to become insulin-producing cells.

Genetics and Genomics: Type 1 diabetes has strong genetic determinants, and studies indicate that more than one gene may influence a person's susceptibility to this disease. Over the last few years, at least 11 genetic markers have been linked to type 1 diabetes. Further identification and mapping of the genes involved will enable researchers to study the functions of the proteins these genes produce. A related field of research is called genomics, in which researchers seek to understand how genes function and how they are expressed in different ways in different individuals. Researchers hope to one day be able to evaluate how multiple genes interact with the environment during the process of disease development and progression in order to find ways to put a halt to type 1 diabetes.

Scientists say it may be many years before all this research leads to a safe and effective cure for type 1 diabetes, although they are encouraged by the significance and the pace of recent research progress. In the meantime, Caroline and her mother have become advocates for finding a cure. Caroline is a delegate to the Children's Congress of the Juvenile Diabetes Research Foundation International on type 1 diabetes. In 1999, she and other children brought the message of type 1 diabetes patients to members of the U.S. Congress to raise awareness of this disease and to ask them to "promise to remember me" when they allocate research dollars. Her mother is chairperson of the 2001 Children's Congress. "Type 1 diabetes is an insidious disease," she says. "Nobody knows what it's like until they live with someone with this disease. Our entire family lives in constant fear of the serious health complications Caroline is subjected to as a result of type 1 diabetes." Caroline, who'd like to become a veterinarian one day, admits that she, too, is frightened of the complications. "I'm very hopeful about the new research that is going on today," says this courageous 10-year-old, "so I don't think I'll let diabetes affect my hopes and dreams."

Diabetes is the leading cause of kidney failure, blindness in adults, and non-traumatic amputations. It also is a major risk factor for heart disease, stroke, nerve diseases, and birth defects, and shortens average life expectancy by up to 15 years.

Type 2 Diabetes in Children: An Emerging Public Health Problem

Type 2 diabetes has traditionally been considered a disease of adults because the age of onset is frequently after age 40 and it is often associated with obesity. Children with diabetes usually have been presumed to have type 1 diabetes; however, in recent years, an increasing number of children who appear with elevated blood glucose levels are being diagnosed with type 2 diabetes. In some studies, the percentage of children with diabetes who have the type 2 form of the disease has risen from less than five percent prior to 1994, to 20-to-30 percent after 1994. Not surprisingly, obesity is one of the major risk factors for type 2 diabetes in children, just as it is in adults. The increase in reports of type 2 diabetes among children parallels a similar rise in the adult population, as obesity has become a major public health concern. In children, the increased incidence of type 2 diabetes appears to be occurring largely in minority populations—Hispanic Americans, African Americans, and Native Americans—again paralleling the disproportionate burden this disease places on the same minority populations in adulthood.

The diagnosis of type 2 diabetes in children is often made because of routine laboratory screenings conducted as part of a school physical, and not because the child has specific health complaints. Thus, many children who do not receive such screening may go undiagnosed until they have symptoms of the disease, at which time they may have been exposed to elevated blood glucose levels for many years and are at risk for developing complications. It is therefore critically important to establish appropriate criteria for screening children, and effective prevention-oriented programs.

Research is needed to establish appropriate and effective treatment regimens for children with type 2 diabetes. Insights may emerge from the ongoing Diabetes Prevention Program (DPP), which is studying the effectiveness of interventions designed to delay or prevent the onset of type 2 diabetes in at-risk adults. However, these interventions may not be the most appropriate strategies for the majority of children with type 2 diabetes, who are in the pre-adolescent or adolescent age range. This age group presents special challenges to health care providers and families when attempting to promote behavior and life-style changes. Prevention and treatment programs must also be tailored to cultural differences that may influence adherence to treatments among racial and ethnic groups. In addition, prevention efforts should recognize that children represent a unique population who may be amenable to population-based, public health interventions through schools or other community organizations.

To combat type 2 diabetes in children, the NIDDK, along with the National Institute of Child Health and Human Development, is supporting research to promote

The photos above show Pima Indian children of Arizona playing and enjoying a nutritious snack of fresh fruit. Public education programs that underscore the importance of preventing obesity through good eating habits such as this, coupled with exercise, may help stem the tide of type 2 diabetes in the Pimas and other minority groups who are disproportionately affected by this disease. It is especially important to prevent obesity in children, whose increasing rates of both obesity and type 2 diabetes are alarming, and, if unabated, may lead to a critical public health problem in the future. Photos: Mr. William Branson, National Institutes of Health.

greater understanding of its causes, to refine diagnostic criteria, to define metabolic abnormalities, and to formulate treatment options. In addition, the NIDDK recently issued two research solicitations for a coordinating center and clinical centers to develop community or school-based primary prevention programs that can be applied in a cost-effective manner to decrease the risk factors for type 2 diabetes and to reduce the incidence of this disease in children and adolescents. Treatment options will be studied to determine the safest, most effective, and most cost-effective strategies to achieve and maintain normal blood glucose levels in the pediatric population.

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Stemming the Tide of Type 2 Diabetes

Two factors play an important role in the growing public health burden of type 2 diabetes—the changing demographics of America in terms of age, ethnicity and race, and the increasing prevalence of obesity, a major risk factor for the disease. Diabetes is the sixth leading cause of death in the U.S., and the third leading cause of death in some minority groups. It places an especially heavy burden on growing segments of the U.S. population—elderly and minority groups. Elderly Americans are especially hard-hit by diabetes—more than 18 percent of adults over age 65 have the disease and many suffer from its complications. Diabetes also disproportionately affects African Americans, Hispanics/Latinos, Native Americans, and Alaska Natives. For example, African Americans are 1.6 times more likely to develop diabetes than Caucasians of similar age. Native Americans and Alaska Natives are 2.8 times more likely to have diagnosed diabetes than non-Hispanic Caucasians of similar age. The NIDDK is spearheading NIH-wide research efforts to combat diabetes and obesity in order to reduce the burden of this devastating disease.

One approach to stemming the tide of type 2 diabetes is to reduce and prevent obesity (see more on obesity on page 55). To this end, the NIDDK has long supported fundamental and clinical research, including the initial discovery of the obesity gene and its protein product, leptin, the major energy regulator, in a mouse model of obesity. This seminal finding has led to the discovery of multiple genes, which control critical aspects of both food intake and energy regulation. The accumulation of new knowledge propelled the discovery of at least five different genetic defects in humans that lead to obesity. Such important research advances have relevance not only to the understanding of obesity, but also to the connection between obesity and diabetes.

The NIDDK supports a National Task Force on Prevention and Treatment of Obesity composed of leading obesity researchers and clinicians. This Task Force evaluates current scientifically-based information about obesity and advises the Institute on important research needs and concepts for future clinical studies. The NIDDK is also exploring the feasibility of forming a Human Obesity Genetics Network to further understanding of the genetic underpinnings of obesity.

A clinical trial of great significance to type 2 diabetes is "Look AHEAD"—Action for Health in Diabetes. This large, multicenter trial is designed to determine whether interventions to produce sustained weight loss in obese individuals with type 2 diabetes will improve health. The trial is expected to recruit a patient population whose overall ethnic and racial composition will reflect the prevalence rates for diabetes in the U.S. The NIDDK is sponsoring this trial along with the National Heart, Lung and Blood Institute, the National Institute of Nursing Research, the National Center for Minority Health and Health Disparities, the NIH Office of Research on Women's Health, and the Centers for Disease Control and Prevention.

Once diagnosed, there are several effective treatment choices for type 2 diabetes. Nutrition and exercise therapy, along with oral medications, used alone, in combination, or with insulin, enable treatment to be tailored to meet an individual's needs. In assessing treatment options, it is important to underscore that the current therapies available for type 2 diabetes are varied and often depend on the stage of the disease. In mild cases or early on in the course of the disease, research has shown that diet, weight loss, and exercise can have beneficial effects. For example, in a group of women identified through the Nurses' Health Study, researchers have found that moderate forms of exercise, such as walking, as well as vigorous forms of activity, such as aerobics, are both associated with a substantial reduction in risk of type 2 diabetes. This finding is a practical approach to prevention because

walking is a form of exercise that is highly accessible, readily adopted, and rarely associated with injury. If lifestyle changes are insufficient to control blood glucose, oral medications may be added to the treatment regimen. Through research, several effective oral drugs have been developed for type 2 diabetes patients; however, some patients may still need to take insulin to control their glucose levels. For patients with type 2 diabetes, good management also means maintaining close control of blood sugar levels throughout the day in order to prevent or delay complications—a regimen that has been conclusively shown to be effective in major clinical trials.

For those at risk for type 2 diabetes, an ongoing NIDDK clinical trial may provide important knowledge about prevention strategies. The Diabetes Prevention Program (DPP) is comparing three regimens: the current conventional advice regarding diet and exercise; a more intensive lifestyle intervention of diet and exercise; and the drug metformin—a medication approved for treatment of type 2 diabetes. The DPP has now completed patient recruitment, with a total of more than 3,800 participants—45 percent of whom are from minority groups. Findings from this study may help advance understanding of the factors that lead to the development of type 2 diabetes and provide evidence that it can be prevented.

Among the programs that are addressing the challenges of diabetes, the National Diabetes Education Program (NDEP) is serving as a catalyst to coordinate and extend prevention efforts through public education. A collaborative initiative of the NIDDK and the Centers for Disease Control and Prevention, the NDEP involves 200 public and private partnerships to promote early diagnosis and improve the treatment and outcomes for individuals with diabetes. The participation of individuals who represent communities of African Americans, Hispanics/Latinos, Native Americans/Alaska Natives, and Asian and Pacific Islanders is a key feature of the program's partnership. The NDEP is currently conducting a series of diabetes awareness campaigns using the theme, "Control Your Diabetes. For Life." This theme is built on the landmark clinical trials that showed the importance of blood glucose control in preventing diabetic complications. By reinforcing this theme, the NDEP encourages patients with diabetes to manage the disease closely in order to live healthier lives. The campaigns target both general audiences and the populations disproportionately affected by diabetes. Television, radio and print public service announcements, educational materials, and information kits for the media and communities are helpful products of the NDEP. The program is currently developing campaigns to encourage health care providers to work with their patients to improve glucose control, and to identify, diagnose, and treat children with type 2 diabetes. The NIDDK is expanding its commitment to the NDEP to develop and promote diabetes messages that reflect the most up-to-date scientific evidence about diabetes control, treatment and prevention. The NIDDK has also featured type 2 diabetes in its Health Disparities Strategic Plan.

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PATIENT PROFILE

Cliff Mitchell – Type 2 Diabetes

Cliff Mitchell was diagnosed with type 2 diabetes at age 60. "What was strange," says Cliff, now 63, "is that a few months prior to my diagnosis I'd had a complete physical and my doctor gave me a clean bill of health." However, because Cliff's mother had diabetes and Cliff was overweight, his doctor cautioned him about the disease. One night, while driving, Cliff's vision started to blur. "I was having trouble seeing the highway signs," he says. He was also experiencing frequent urination, which he attributed to drinking lots of water. The next night, while watching a football game on TV, Cliff realized he couldn't read the words scrolling across the bottom of the screen. He began to get worried, so the following day he went to an eye doctor who sold him a pair of glasses—which, it turns out, Cliff didn't need. But the optometrist, suspecting diabetes as a cause for Cliff's blurred vision, also advised him to follow up with another visit to his physician. A blood test confirmed that Cliff's blood sugar, or glucose level, was high and that he had type 2 diabetes. Last year, Cliff's oldest daughter, age 37, also was diagnosed with the disease.

Type 2 diabetes is the most common form of the disease, accounting for 90 to 95 percent of diagnosed diabetes cases. Unlike patients with type 1 diabetes, in which the body's immune system destroys the insulin-producing cells required to build and store energy, most people with type 2 diabetes do not require daily injections of insulin to control their glucose levels in order to survive. In fact, most people with type 2 diabetes are able to control the disease with diet and exercise. However, patients face the prospect of developing one or more serious complications as a result of the disease.

As a result of research conducted by the NIDDK, scientists are beginning to gain an increased understanding of the genetic underpinnings of this complex disease. This progress is expected to lead eventually to new therapies, more effective prevention efforts, and ultimately, a cure for type 2 diabetes.

Cliff Mitchell controls his blood sugar levels through a combination of medication and lifestyle changes. Still, he admits that "managing" his disease is a sometimes difficult task. Research supported by NIDDK is leading to new therapies and seeking to uncover the genetic causes of this complex disease.

In addition, public awareness and education programs are aimed at reducing the death and disability associated with diabetes and its complications. For example, individuals who are obese or are of African American, Hispanic American or Native American heritage are particularly at risk for this disease. The NIDDK and other organizations are working to alert at-risk populations about these facts and about what they can do to help control their weight and to manage this disease.

Living with the Disease

Because type 2 diabetes most often can be controlled by diet and exercise, it is sometimes referred to as a "manageable disease." However, managing type 2 diabetes is a daily struggle for many, including Cliff. "When I was first diagnosed, I started exercising by walking and swimming three or four times a week, cut back on eating sweets, and stopped drinking alcohol, which for me amounted to about one or two drinks a week." He also tested his glucose levels two or three times a day by pricking his finger and using a sugar testing machine, called a meter. But as time passed, Cliff became less diligent. "Now I'm having trouble controlling my weight because I'm not exercising as much as I

should," says Cliff, who currently weighs 280 pounds and stands six-feet three-inches tall. The fact that Cliff's wife is a gourmet cook doesn't make controlling his weight any easier, and he's not testing his glucose levels as regularly as he did when he was diagnosed. "I'm testing only when I feel bad," he says, meaning when his eyesight begins to get fuzzy, or when he starts feeling unusually tired or starts urinating frequently. To help control his glucose levels, Cliff takes diabetes medication.

Diabetes costs the nation nearly $100 billion annually in direct and indirect costs, including disability, work loss, and premature death.

But Cliff must remain cautious. The fact that he is African American puts him at increased risk for devastating complications of diabetes that can be fatal.

For example, type 2 diabetes can lead to:

- *Kidney disease*, including kidney failure, also referred to as end-stage renal disease (ESRD).
- *Diabetic eye disease*, which can lead to impaired vision and ultimately to blindness.
- *Diabetic nerve disorders*, including pain or loss of sensation in the feet and hands, which can lead to amputations, as well as diseases involving bodily functions, such as digestion and bowel movements.
- *Macrovascular disease*, which can lead to heart attacks and strokes.

TYPE 2 DIABETES FACTS

- **Accounts for 90 to 95 percent of the 16 million people in the U.S. with diabetes.**
- **Leading cause of new blindness, end-stage renal disease, and non-traumatic leg amputations.**
- **Major risk factor for heart disease and stroke.**
- **Shortens average life expectancy by up to 15 years.**

Two clinical trials supported by the NIDDK—the "Diabetes Control and Complications Trial (DCCT)," and the "United Kingdom Prospective Diabetes Study (UKPDS)"—demonstrated clearly that close control of blood sugar can prevent or reduce diabetes complications. This is good news for Cliff and millions of others who suffer from type 2 diabetes because, in recent years, as a result of basic and clinical research, several oral medications are now available that work to control the level of glucose in the blood.

What's Being Done

Type 2 diabetes is reaching epidemic proportions in the U.S., most recently among children from minority groups, with obesity being a major risk factor. Unfortunately, most people are unaware that obesity and racial/ethnic background put them at high risk for this disease, and many are unfamiliar with the warning signs of diabetes. Much is being done to generate new knowledge about this disease—which will lead to better treatment and prevention strategies—and to translate new findings to the public and to medical practice. For example, the NIDDK is currently:

- Supporting the multicenter "Diabetes Prevention Program" (DPP) clinical trial to determine whether lifestyle and pharmacologic interventions can prevent or delay the onset of type 2 diabetes.
- Supporting a clinical trial called "Look AHEAD"—Action for Health in Diabetes, designed to determine whether interventions to produce sustained weight loss in obese individuals with type 2 diabetes will improve health.
- Supporting a National Task Force on the Prevention and Treatment of Obesity, as well as several Obesity/Nutrition Research Centers to elucidate the genetic and metabolic underpinnings of obesity.
- Spearheading the National Diabetes Education Program (NDEP) with the co-sponsorship of the Centers for Disease Control and Prevention (CDC) and 200 partner organizations. The NDEP is conducting ongoing diabetes awareness and educa-

tion activities for people with diabetes and their families. Special efforts are being made to address the needs of minority populations disproportionately affected by type 2 diabetes.

- Supporting the "Weight-Control Information Network" (WIN) to disseminate science-based information about obesity.

The Impact of Research on the Genetics of Diabetes

In addition, research on the genetics of diabetes is greatly increasing understanding of the disease. For example:

- Progress has been made in identifying specific genes involved in relatively rare subtypes of type 2 diabetes, such as Maturity Onset Diabetes of the Young (MODY), and researchers are continuing to search for other disease-causing genes.

- Studies have found that a gene, *calpain-10*, appears to increase susceptibility to type 2 diabetes in certain Mexican American and Northern European populations. This gene appears to be the gene on chromosome 2 that was first linked to susceptibility to type 2 diabetes and termed *NIDDM1*.

- Based on genetic studies, researchers believe that some racial and ethnic groups at very high risk for type 2 diabetes may only develop the disease if certain environmental factors "trigger" their genetic predisposition.

- Researchers are intensely investigating defects in genes that contribute to obesity in rodents, and their roles in human obesity. A major breakthrough in this area was the discovery of an obesity gene in mice and the hormone it produces, called leptin. This hormone is secreted by fat cells and acts on cells in the brain and in tissues to help regulate food intake and energy balance.

Although type 2 diabetes affects all segments of the population, it is particularly prevalent in African Americans, Hispanic Americans, Native Americans and Alaska Natives, as well as some Asian Americans and Pacific Islanders.

- To capitalize on new tools of molecular genetics, the NIDDK supports a Type 2 Diabetes Genetics Consortium. The Consortium is expected to accelerate research momentum and advances with respect to genetic factors underlying type 2 diabetes.

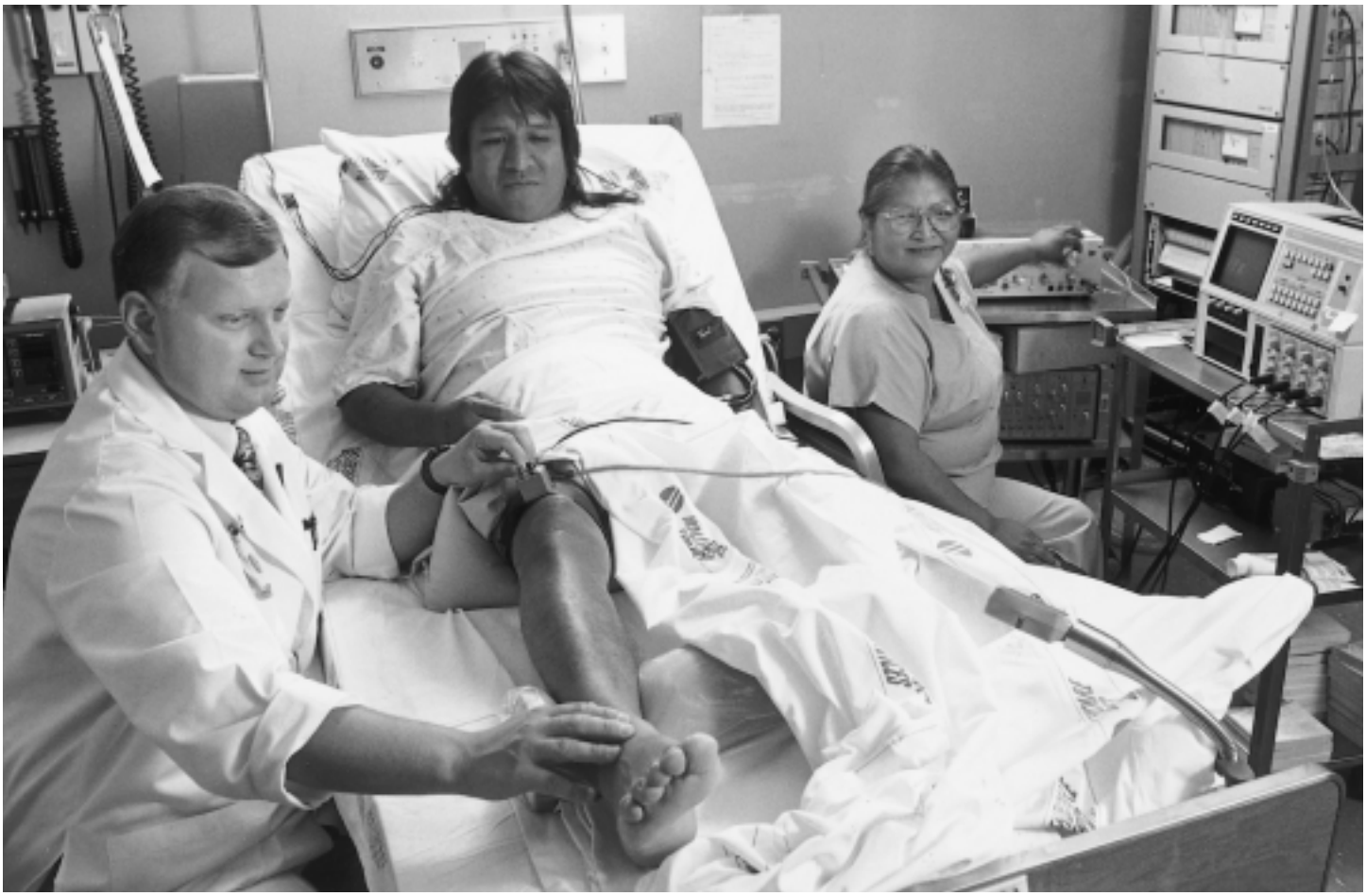
Although impressive progress has been made, much more research is still needed at the molecular level to help people with type 2 diabetes. In the meantime, Cliff and his daughter will need to manage their blood sugar levels vigilantly to avoid or minimize the complications of the disease.

To help others with the disease, Cliff is a member of the National Diabetes Education Program (NDEP) African American Task Force. He assists in the development of culturally relevant media campaigns, which include public service announcements, posters, and other literature targeting the African American community.

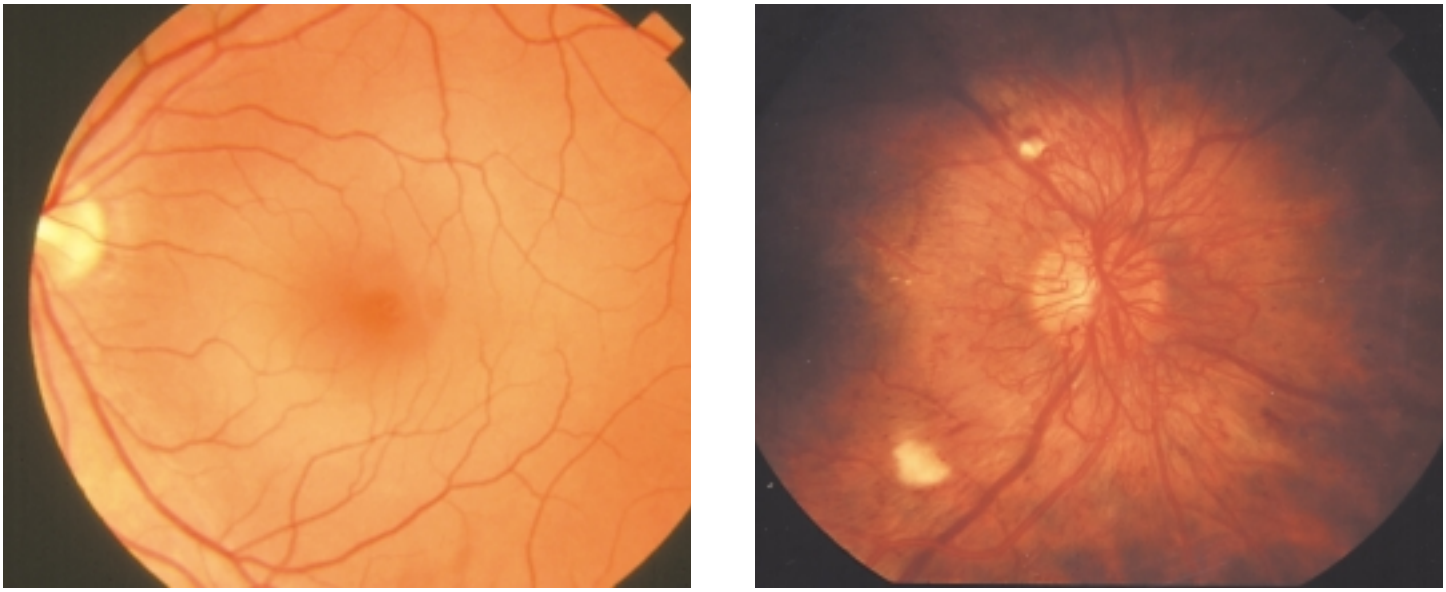
Complications of Diabetes: Preventing Damage to Cells

Complementing the search for genes underlying diabetic complications, the NIDDK supports extensive research on cell biology. Both type 1 and type 2 diabetes cause widespread damage to blood vessels throughout the body. These complications are responsible for most of the illness and death associated with both forms of diabetes. Understanding how complications occur and progress at the cellular level is therefore paramount to improving treatment, and ultimately, to attaining the means to prevent these devastating consequences of diabetes.

New insights about diabetes complications are being gleaned from an ongoing study, the Epidemiology of Diabetes Intervention and Complications (EDIC). This study is a long-term follow-up of patients who participated in the NIDDK's landmark Diabetes Control and Complications Trial (DCCT)—a multicenter clinical trial of over 1,400 individuals with type 1 diabetes. The DCCT found that maintaining blood glucose levels as close to normal as possible through intensive therapy was significantly more beneficial than conventional therapy in preventing or delaying the development of eye, kidney and nerve complications associated with diabetes. In the EDIC, researchers are continuing to use the same blood test, called an HbA1C test. This test measures the degree to which hemoglobin, a red blood cell protein, is modified by glucose. The HbA1C test is an excellent, integrated measure of blood glucose control over periods of weeks to months. Thus far, the EDIC study has shown that the marked reduction in the risk of progressive eye and kidney disease in those who received intensive therapy during the DCCT has been sustained for at least four years—even though some increases in HbA1C levels have been observed. These findings are important for the medical management of diabetes because they underscore the value of early initiation of intensive therapy, continued for as long as possible, in producing long-term



Diabetes is a major contributing factor to heart disease and stroke and is the leading cause of kidney failure, blindness, and amputation in adults. It is very important for patients with diabetes to receive thorough medical checkups. Photo: Mr. William L. Branson, National Institutes of Health.



Diabetic retinopathy, damage to the tissue that lines the back of the inside of the eyeball, is the most common form of diabetic eye disease. In retinopathy, the small blood vessels that supply the retina with oxygen and nutrients are damaged and, as a consequence, sight is impaired. The photographs above contrast a normal, healthy retina (left) with one exhibiting retinopathy (right). Note the increased density of blood vessels in the diseased eye. Photo: National Eye Institute, National Institutes of Health.

beneficial effects with respect to the eye and kidney complications resulting from diabetes. Also reinforcing and extending the findings of the DCCT was another study conducted in type 2 diabetes. That clinical trial, the United Kingdom Prospective Diabetes Study (UKPDS), also demonstrated the preventive effects of intensive therapy. In addition, the trial showed that good blood pressure control produced a major benefit in decreasing complications of the large blood vessels. Although close control of blood glucose levels has been proven to reduce the risk of developing complications of diabetes, the risk of lowering blood glucose too low is very real. This risk is a major concern in those individuals with type 1 diabetes. A recent workshop focused on this issue with the support of the Juvenile Diabetes Research Foundation International, the American Diabetes Association, the National Aeronautics and Space Administration, the NIDDK, the National Institute of Neurological Disorders and Stroke, and the National Institute of Child Health and Human Development. Participants discussed the effects of low blood glucose on the brain. It is expected that research initiatives will be forthcoming based on the recommendations of the scientists and representatives of voluntary organizations attending.

While clinical research has demonstrated that close control of blood glucose reduces the risk of complications, basic research has shown that—even before the clinical signs of diabetic complications appear—damage has already occurred at the cellular level. Elevated blood glucose levels may cause damage to the endothelial cells that line the blood vessels. This damage may, in turn, lead to abnormalities in the large and small blood vessels. Proteins bound on the surface of endothelial cells are a target for modification by glucose. When modified, these proteins trigger a cascade of events in the immune system leading to abnormal growth of endothelial cells in the vessel wall. This proliferative growth is characteristic of the abnormalities seen in diabetic complications.

Cellular damage leading to complications may also result from high levels of an unusual form of oxygen, known as superoxide, caused by elevated blood glucose. Superoxide is extremely unstable and can damage a large number of components within cells, including the mitochondria—the key to energy utilization. NIDDK-supported researchers have shown that, when levels of superoxide in the mitochondria are reduced, four separate biochemical pathways implicated in the development of complications will be less active, thereby reducing the cellular damage resulting from elevated blood glucose levels.

One of the most severe complications of diabetes is kidney failure or end-stage renal disease. Diabetes is the most common cause of end-stage renal disease in the U.S. In individuals with diabetes, even small amounts of pro-

tein in the urine increase the risk of progressive kidney disease. Researchers report that, in mice with diabetes, kidney failure is caused by over-production of a protein called transforming growth factor-beta. This protein causes scar tissue to form in the kidneys, which eventually interferes with kidney function. However, when this protein is neutralized with an antibody against it, kidney failure does not develop. This is the first proof-of-concept study showing that transforming growth factor-beta may cause end-stage renal disease.

The NIDDK is undertaking several new and expanded initiatives designed to further understanding of the key mechanisms involved in development of the complications of diabetes and the means to reduce or prevent them. Several initiatives involve productive collaborations with other NIH components whose research missions focus on organs and systems adversely affected by diabetes, such as the National Heart, Lung and Blood Institute for the cardiovascular complications; the National Institute of Neurologic Disorders and Stroke for diabetic neuropathy; and the National Eye Institute for diabetic retinopathy.

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Metabolic Issues in the Treatment of AIDS

Research supported by the NIDDK has formed the basis for increased understanding of the development of the AIDS Wasting Syndrome and has paved the way for a number of pilot studies of therapeutic interventions. The AIDS Wasting Syndrome is an involuntary loss of more than 10 percent of body weight accompanied by other symptoms. It may result from low food intake, poor nutrient absorption and altered metabolism. While an important international health concern, the incidence of wasting in AIDS patients in the U.S. has declined with the introduction of highly active anti-retroviral therapies (HAART). The HAART regimen includes at least three anti-retroviral drugs and usually at least one protease inhibitor, a type of drug that suppresses replication of the human immunodeficiency virus, HIV. The enhanced effects of these drugs, when used in combination with other drug therapy, have resulted in significantly reduced plasma levels of HIV and reduced mortality and morbidity associated with HIV infection.

Recent evidence indicates that, although HIV may not be detectable in a patient's blood, some of the virus may still exist in the body. Even after protease inhibitor treatment for up to two years, virus particles can still be detected in some cells. These findings have led to the present recommendations that protease inhibitor treatment be continued to suppress viral infection. In order to deplete latent stores of HIV, treatment may need to be maintained for up to ten years and possibly longer. Given the increased life expectancy of HIV-infected individuals and the prospect of long-term HAART therapy, recent reports of endocrine, metabolic and body composition changes in this population are raising a significant health concern.

Reports have implicated protease inhibitors in causing abnormal fat redistribution (lipodystrophy), insulin resistance, increased blood lipid levels, and in some cases, development of type 2 diabetes. Animals with an absence,

or low levels, of a protein that facilitates transport and storage of glucose in fat and muscle cells are unable to store fat, respond poorly to insulin, and are more likely to develop diabetes. NIDDK-supported investigators found that human fat cells absorbed less glucose after exposure to protease inhibitors, and that the drugs acted directly to prevent the transport protein from functioning. These results suggest that HIV protease inhibitors block the body's ability to store glucose, making individuals treated with these agents more likely to develop type 2 diabetes. This research points to a need to develop new agents to treat HIV without increasing the risk of diabetes.

In studies of AIDS patients with lipodystrophy and abnormal glucose metabolism, researchers have demonstrated that low-dosage treatment with metformin—an insulin-sensitizing agent—reduced insulin resistance and improved weight and blood pressure. Though further studies are needed to determine the long-term benefits of insulin-sensitizing agents, therapies can now be developed that improve insulin resistance and fat redistribution, yet permit AIDS patients to be maintained on their other medications, further extending their long-term prognosis.

To determine the extent of the side effects of protease inhibitor therapy, the NIDDK is supporting a nationwide multicenter study in collaboration with the National Institute of Allergy and Infectious Diseases and the NIH Office of AIDS Research. Because minority individuals from Hispanic and African American backgrounds are already disproportionately affected by HIV, and are also at greater risk for diabetes and cardiovascular disease, the consequences of metabolic complications resulting from protease inhibitors may be particularly devastating in these populations. The new collaborative study, which includes many sites across the country, thus has a high target for recruitment of patients from minority groups. This study should provide a strong scientific basis for the development of potential therapies, as well as for the design of appropriate research to elucidate the underlying causes of metabolic abnormalities associated with long-term treatment of AIDS patients with protease inhibitors.

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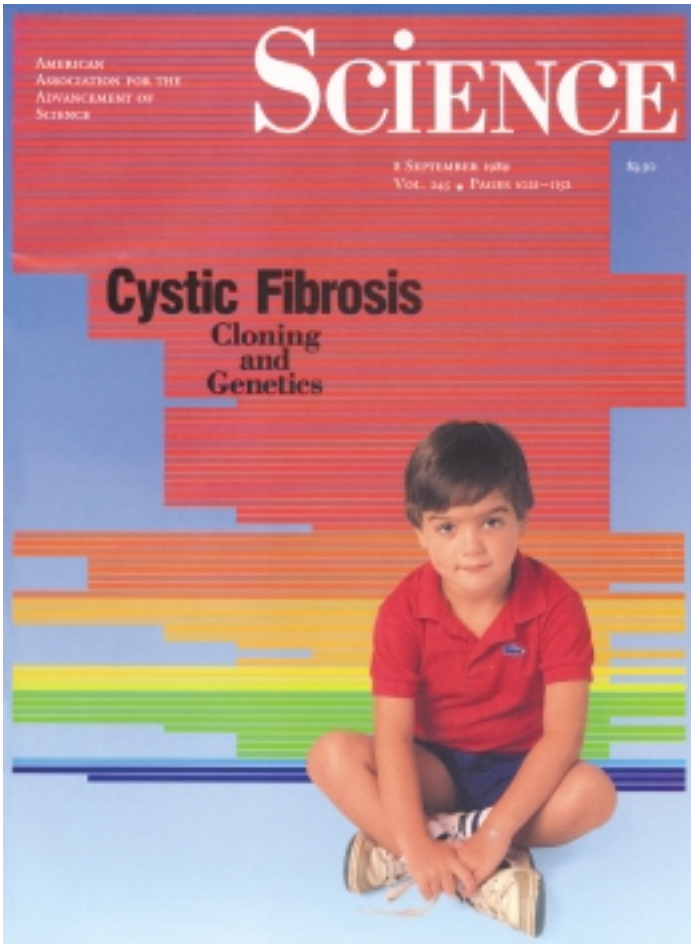
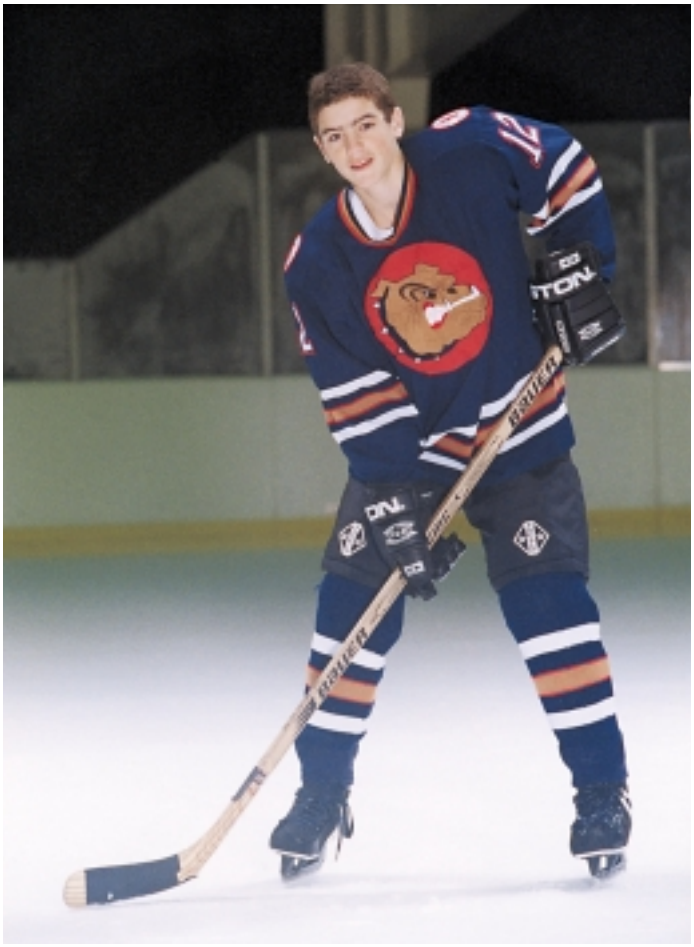
Cystic Fibrosis

The cover of the September 1989 issue of the journal *Science* features a young child, Danny Bessette, who has inherited the genetic disease, cystic fibrosis (CF). Behind him are colored bars representing the chromosomes of other patients with CF. The simple title, "Cystic Fibrosis—Cloning and Genetics," announced the historic discovery of the gene responsible for most forms of this disease. Inside the journal, three articles described the innovative research leading to this landmark achievement. The discovery of the CF gene enabled accelerated research and thus offered hope of a brighter future for children and young adults with this disease. The dedicated researchers who attained this achievement in genetics were supported by the NIDDK, as well as other private and public institutions.

Cystic fibrosis (CF) is a genetic disease affecting 30,000 Americans. It is diagnosed in approximately 2,000 children born each year. Because it is a recessive genetic trait, children with the disease must have inherited a defective copy of the gene from both parents. It is now estimated that there are over 10 million individuals in the U.S. with one copy of the mutant gene. If a child's parents both have the gene, the child has a one in four chance of inheriting CF. With the discovery of the CF gene, scientists were able to find the protein whose production the gene controls—the CFTR protein. This protein is found in the membranes of secretory cells. There, it functions as a chloride ion channel, regulating the flow of chloride and other molecules out of the cell. For individuals with CF, proper regulation of chloride is not possible. Either the protein channel is degraded before it reaches the cell membrane, so that there is an insufficient number of channels for the release of chloride, or the channels are defective and unable to function normally. Absence of this channel affects the mucus glands of the body, resulting in serious breathing and digestive problems. Inadequate secretion of chloride from cells in the lungs results in the formation of a thick mucus. This is exacerbated when immune system cells, summoned to the lungs to alleviate the distress, die off and release their viscous DNA. Because of these conditions, CF patients are vulnerable to repeated infections that cause permanent damage to the lungs and eventually can result in death. CF affects the sweat glands, the digestive and reproductive systems, and the respiratory system. One of the symptoms



Danny Bessette, at age 5, is the child with cystic fibrosis featured on the cover of the landmark September 8, 1989 issue of the journal *Science*. This issue contains articles on the discovery of the cystic fibrosis gene. Photo: The American Association for the Advancement of Science.



Danny Bessette is shown playing hockey at age 14. Danny is now 16 and continues to enjoy hockey as captain of his high school team. Advances in research have helped Danny to enjoy the life of an active teenager. Photo: The Bessette family.

of CF is very salty sweat caused by poor absorption of salt in the sweat ducts. Forty years ago, an NIDDK scientist took advantage of this characteristic to develop a simple sweat test for identifying patients with CF. The sweat test is still the primary test used by physicians today to screen individuals for CF. If a patient's results are questionable, then additional, more complex tests are used to clarify the diagnosis.

The discovery of the CF gene not only enabled identification of the CFTR protein, but also paved the way to development of a mouse model for CF. The model was generated using a technique known as gene targeting. In these experiments, the CFTR sequence was interrupted by the insertion of another gene. This procedure generated mice with truncated CFTR proteins and with the most common mutation found in patients with CF. The gene that codes for a protein will determine the order of the specific amino-acid building blocks the protein will contain. The CFTR protein is composed of 1,476 amino acids, and 70 percent of CF cases are caused by a deletion known as delta F508 in the patients' DNA. As a result of the mutation, only one amino acid is deleted—a phenylalanine in position 508—which results in this devastating disease.

A recent study used the CF mouse model to investigate a previously reported deficiency in the lipid (fatty acid) metabolism of patients. The aim of this study was to determine whether the deficiency exists in all organs or only in those clinically affected by the disease, and whether the deficiency plays a critical role in the clinical expression of the CF gene. The fatty acid imbalance that results from CF is characterized by alterations in the levels of two fatty acids, an increase in AA:omega-3 fatty acid and a decrease in DHA:omega-6 fatty acid. A significant imbalance in membrane fatty acid was detected in CF mouse organs where evidence of disease is found—the

lungs, pancreas and ileum; however, no significant change in fatty acids was found in the brain and kidney, which do not exhibit disease characteristics. Based on these results, researchers administered oral DHA to the mice as a potential treatment. Oral DHA corrected lipid imbalances in CF mice and reversed visible expression of the disease. This work demonstrates that membrane lipid metabolism plays a central role in the disease process of CF and offers a specific therapeutic target and a potential new treatment.

In another study, scientists investigated the mechanisms that control the CFTR channel. It is well known that molecules known as ATP release energy when degraded, thus making energy available as fuel for cell maintenance and function. Researchers were aware that the CF channel required this molecule for energy to regulate the flow of chloride from the cell, and they wanted to determine the underlying molecular mechanisms involved. Using a technique known as "yeast two-hybrid analysis," they were able to identify a protein, AMPK, that interacts strongly with the CFTR protein. In additional experiments, they determined the genetic regions and specific amino acids involved in the interaction for both proteins. They then expressed these proteins using a frog embryo system and determined that CFTR and AMPK were co-expressed. When these proteins were expressed in cells that line the nasal passages of the rat, both proteins were located in the area of the cells where effluent is released. AMPK was also shown to modify the CFTR protein by a process known as phosphorylation—the addition of phosphate molecules. This process reduced the ion channel's activity, inhibiting chloride flow by 35 to 50 percent. The protein AMPK acts as a metabolic sensor in cells by responding to fluctuations in cellular ATP. It is likely that the regulation of ATP is mediated by AMPK's "sensing" the cellular concentration of ATP and responding by modifying the CFTR protein, thus reducing ion channel activity. The inhibition of CFTR by AMPK may prove to be an important mechanism that closes the channel under conditions of metabolic stress when ATP concentrations are low; thereby conserving cellular fuel and linking the metabolic state of the cell to the transport of molecules from the cell.

These advances in research on CF and other genetic diseases would not have been possible without the discovery of the structure of DNA; the elucidation of the genetic code and its use as a template in protein synthesis; the identification of enzymes that cut DNA at specific locations; and the development of a host of other new technologies based on these achievements. Since discovery of the CF gene, scientists have continued to build on the ever-expanding body of knowledge about this disease. They are coming to an increased understanding of how the deletion of a single amino acid, as in the delta F508 mutation, or the absence of the CFTR protein, caused by other mutations, can result in such a debilitating and deadly disease. Prospects have improved for children with CF, represented by Danny Bessette on the 1989 cover of *Science*. Through research, life expectancy and quality of life are better, and new insights into the genetic and metabolic underpinnings of the disease are being gained. CF—about which researchers had no real clues—is now beginning to yield to powerful research tools.

The NIDDK maintains strong support for basic and clinical research studies of CF. Cystic Fibrosis Core Centers provide shared resources to enhance research ranging from defining the molecular processes of CF to the development of new therapies for the disease, including gene transfer approaches, the development of animal models and delivery systems for the development of CF treatments, clinical assays, and inflammation studies.

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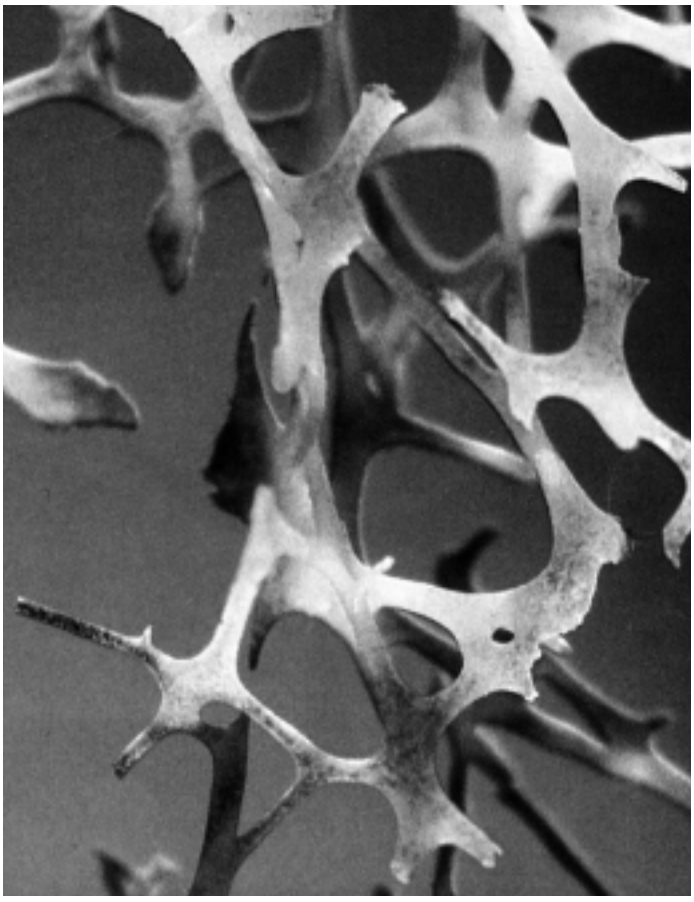
Excess Calcium: A Culprit in Bone and Kidney Disease

When the body produces excessive parathyroid hormone, blood calcium levels rise, a condition referred to as hypercalcemia. In part, this increased blood calcium is due to the abnormal transfer of calcium from the bones to the blood, which may weaken the bones, cause osteoporosis, and lead to bone fractures. Calcium levels may also increase in the urine, with resultant kidney stones.

Production of excessive parathyroid hormone is called hyperparathyroidism (HPT). It usually occurs from a benign tumor on the parathyroid gland. When produced in normal quantities, this hormone maintains the correct balance of calcium and phosphorous in the body by regulating the release of calcium from bone, absorption of calcium in the intestine, and excretion of calcium in the urine.

Hyperparathyroidism affects about 100,000 Americans each year. Affected women outnumber men by two to one, and the risk increases with age. It is being diagnosed increasingly in patients who have few or no symptoms. The diagnosis is usually based on an unexpected rise in blood calcium level found incidentally as the result of a routine blood test. Surgery to remove the parathyroid gland is currently the only treatment for this disorder and it almost always solves the problem.

A clinical research question has been whether asymptomatic patients should undergo surgery. Recently, NIDDK-supported scientists reported that surgical removal of the affected parathyroid gland(s) can provide significant benefits for patients with asymptomatic or mild disease. They studied the clinical course of over 100 patients with primary disease, most of whom had no symptoms. Over a period of ten years, most of the asymptomatic patients who did not undergo surgery remained stable. None developed kidney stones, loss of kidney function, bone fractures, or life-threatening high levels of calcium. However, 27 percent of these patients did have clinical progression, as defined by increases in blood or urine calcium levels or decreased bone density. In the asymptomatic patients who had surgery, substantial and sustained increases in bone density were observed.



This photo shows bone affected by osteoporosis. This disease causes a marked decrease in bone density. Consequently, the bone becomes fragile and subject to a high risk of frequent fractures. Photo: Dr. Robert Lindsay, Helen Hayes Hospital, West Haverstraw, New York.

These results revealed that surgery provides many patients who have mild or asymptomatic hyperparathyroidism with a significant, rapid, and sustained improvement in bone density and other benefits. Surgery has become a safe, simple and rapid treatment for this disease. Now, abnormal glands may be identified prior to surgery through the use of advanced imaging tests. Also, rapid measurement of parathyroid hormone during surgery provides timely information on whether the abnormal gland(s) has been successfully removed. These new technologies can improve the success of surgery in curing the disease.

The NIDDK continues to support research on parathyroid hormone and related factors, and on basic bone biology. Results of these studies will provide insights into the mechanisms by which bone regulatory hormones act at the cellular level. These insights are critical to an understanding of the events that regulate bone remodeling and, ultimately, will contribute to the development of effective therapies for bone and bone-related diseases, including osteoporosis and hyperparathyroidism.

Note: It is of particular research and clinical interest that, while sustained parathyroid hormone increases cause bone loss, intermittent parathyroid hormone elevation can cause a net *gain* in bone density. This difference is important as it relates to possible alternative treatments for osteoporosis (see the following "Story of Discovery" on osteoporosis).

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From Bench to Bone – Basic Research Yields Osteoporosis Treatments

An album of family photos documents the transformation of a beautiful young lady of 20 into a hunched and deformed woman of 60. A tucked-away letter to her daughter reads: "My daily life has changed completely. I walk with two canes. I can't bend down and I am in constant pain. I cannot carry or pick things up, and therefore I cannot do my own shopping. All my life I have been active, now I can't even do routine activities."

The human costs of osteoporosis are enormous. This disease is characterized by low bone mass and bone deterioration. A comprehensive treatment program includes a focus on proper nutrition, exercise, and safety measures to prevent falls that may result in fractures. In addition, medication may be prescribed that will slow or stop bone loss and increase bone density. If left untreated, the bones of a person with osteoporosis become weak and fragile, leading to an increased risk of fractures. All bones may become brittle, but fractures to the hip, spine and wrist occur most often and can lead to prolonged or permanent disability, even death. According to the National Osteoporosis Foundation, approximately 10 million people in the U.S. have osteoporosis and about 18 million more have low bone density, placing them at increased risk for developing the disease. Osteoporosis has been reported in people of all ethnic backgrounds and the chances of developing osteoporosis are four times greater in women. Direct and indirect expenditures for osteoporosis and related fractures are estimated at $14 billion each year, and the cost is rising.

Although there is no cure for osteoporosis, major strides have been made in its treatment during the past 30 years. Several new classes of agents that help to stop bone loss and prevent further fractures have been approved by the Food and Drug Administration for marketing in the U.S. Bone cells have been shown to respond to the female hormone estrogen, which acts to slow down the removal of calcium from bone. Unfortunately, estrogen, when taken alone, has been shown in some studies to slightly increase a woman's risk of developing breast and endometrial cancer. SERMs, or selective estrogen receptor modulators, were therefore developed to maximize the beneficial effects of estrogen on bone, while minimizing the adverse effects on other organs and tissues. Bisphosphonates are agents of another class that specifically target bone and act to reduce bone loss by mimicking the action of estrogen. Research on the regulation of bone development and remodeling has played a pivotal role in fueling the major scientific advances that have expanded knowledge about the development and progression of osteoporosis and that are leading to newer and more effective strategies for its treatment and prevention.

Bone is a living, growing tissue. It is made mostly of collagen, a protein that provides an elastic framework, and calcium phosphate, a mineral that adds strength and serves to harden the framework. One key hormone responsible for regulating the cells involved in bone formation—the osteoblasts—is parathyroid hormone (PTH). Paradoxically, PTH exhibits both anabolic and catabolic effects—that is, it has been shown to control the transfer of calcium both into and out of bone. One of the earliest accomplishments in bone research was the isolation and purification of PTH, which contributed to the collaborative development of a novel radioimmunoassay to measure PTH levels in the blood. Using the radioimmunoassay, researchers could then study the factors governing PTH secretion. Resulting data clearly demonstrated that changes in serum calcium, in turn, controlled PTH secretion. The isolation and purification of PTH paved the way for further studies on the synthesis of recombinant PTH and on the mechanisms of action of this hormone.

A few years later, researchers announced the successful identification, cloning and sequencing of the cellular receptor for PTH in several species, including rat, mouse and humans. Although the receptor's overall structure greatly resembled that of other trans-

membrane peptide hormone receptors, it differed sufficiently so as to constitute a major new receptor subfamily. Once the structure was deduced, it was then possible to analyze its molecular mechanisms of action. Such knowledge is crucial to understanding the processes that lead to weakened bones and to the development of hormone-based therapies.

It had long been known that the amount of circulating calcium played a role in regulating the amount of PTH secreted. Yet, until recently, the exact mechanisms that permitted the cells of the parathyroid gland to secrete hormone in response to calcium levels remained elusive. Impressive studies led to the identification and cloning of a calcium-sensing receptor, defining an important step in the regulatory pathway of calcium-responsive PTH secretion. This receptor acts to "sense" extracellular levels of calcium and "signals" the parathyroid gland to regulate levels of PTH secretion. Different parts of the calcium-sensing receptor structure were shown to mediate different parathyroid-gland signaling pathways, explaining how secreted PTH can at times signal the osteoblast to increase bone mineral, and at other times, signal the osteoclast—a cell involved in the breakdown of bone—to reduce bone mineral. Thus, this research helped to explain the mystery of the anabolic and catabolic effects of PTH. The long-term implications of this work suggested the possibility of not only designing therapeutic agents that would suppress the signaling events leading to bone loss, but also of devising agents that would stimulate the formation of bone, such as synthetic PTH.

An understanding of the anabolic actions of hormones such as PTH led to the design of two small-scale clinical trials testing the efficacy of synthetic PTH as a treatment for osteoporosis. Results demonstrated a beneficial effect on increasing spinal bone mineral density and preventing bone loss from the hip and total body in young women. Synthetic PTH is also proving beneficial in treating a severe form of osteoporosis caused by glucocorticoid hormones, such as prednisone, which are used to treat inflammation. An ongoing clinical study is evaluating the effect of daily administration of synthetic PTH on the risk of fractures in postmenopausal women who have glucocorticoid-induced osteoporosis and are currently receiving estrogen replacement therapy. Preliminary results indicate a greatly reduced risk of spine fractures and non-traumatic, non-spine fractures within one to two years of beginning therapy. Other recent research has shed light on how estrogen works to maintain bone mass by shortening the lifespan of the cells that are responsible for resorption of bone mineral. It does so indirectly, by stimulating the production and release of a potent growth factor in bone. In the absence of estrogen, this control is lost. The osteoclasts are no longer properly regulated, resulting in excessive bone loss.

Though no significant side effects have been attributed to PTH therapy, current treatment requires painful injections, which the patients must perform daily. Thus, the development of an oral agent that exhibits few side effects would provide a valuable treatment alternative. A number of studies are under way on novel oral agents that enhance PTH secretion in animal models. These agents act through the calcium-sensing receptor, which allows PTH secretion to be regulated independently of calcium levels in the blood. Preliminary results from a few studies have demonstrated a dramatic decrease in the kind of bone turnover that results in excessive loss of bone mineral, thus suggesting additional potential therapeutic agents for the future treatment and prevention of osteoporosis and related bone disorders.

The long-term investments in studies of the underlying mechanisms of bone loss and bone remodeling, as well as the development of cellular and animal models to study these processes, have enabled the development of new drugs to treat osteoporosis. These approaches have laid the foundation for understanding the mechanisms of action of these agents and for the design of safe and more effective therapies. These discoveries open exciting and promising new avenues for future exploration and provide more options for the treatment of this disease. A research imperative is to capitalize on these discoveries to develop newer, safer and more effective compounds to prevent and treat osteoporosis. With translation of these novel treatments to medical practice, it will become possible to prevent bone loss, reduce the incidence of fractures, and improve the overall quality of life for patients at risk for osteoporosis.