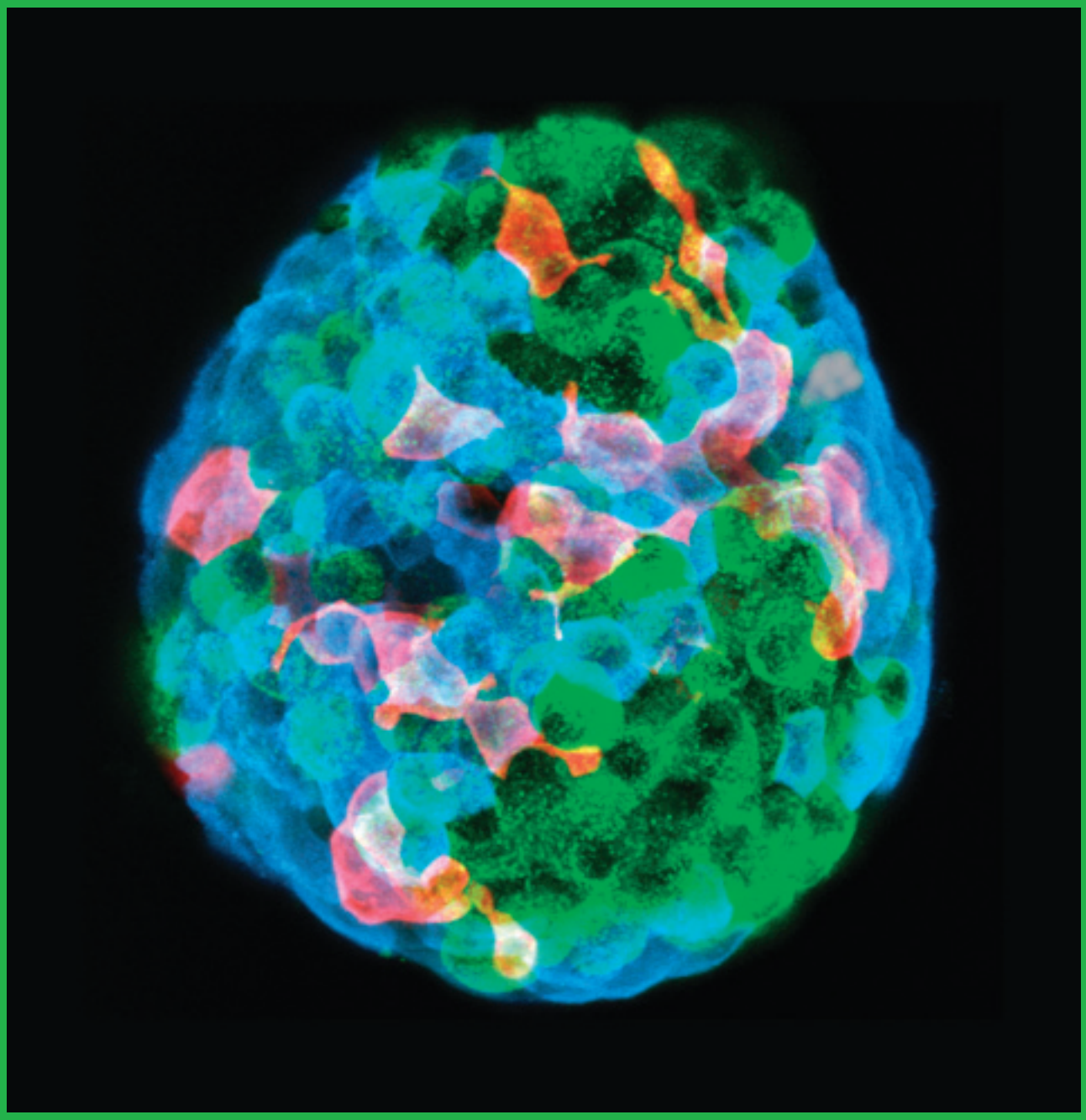

An islet—Islets are clusters of cells within the pancreas containing the insulin-producing beta cells critically important in diabetes. This islet is labeled to show beta cells, which produce insulin, in green; alpha cells, which produce glucagon, in blue; and delta cells, which produce somatostatin,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

Diabetes, Endocrinology and Metabolic Diseases

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

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

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

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

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

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

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

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

BLOOD SUGAR CONTROL IN TYPE 1 DIABETES: A BALANCING ACT

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

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

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

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

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

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

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

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

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

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

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INJECTED INSULIN DOES NOT DELAY ONSET OF TYPE 1 DIABETES

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

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

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

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

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

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

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

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FACING DOWN AUTOIMMUNITY IN TYPE 1 DIABETES PREVENTION AND TREATMENT

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

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

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

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

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

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

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

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CARDIOVASCULAR DISEASE AND DIABETES

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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GENES DIRECTING PANCREATIC DEVELOPMENT

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

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

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

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

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TECHNOLOGY IN DIABETES RESEARCH

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

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

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

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

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

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

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

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

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DEVELOPMENT OF NEW TECHNOLOGIES FOR THE TREATMENT OF CYSTIC FIBROSIS

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

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

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

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

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

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

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GENE THERAPY IN DOGS WITH MUCOPOLYSACCHARIDOSIS VII (MPS VII)

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

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

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

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

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

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

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

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

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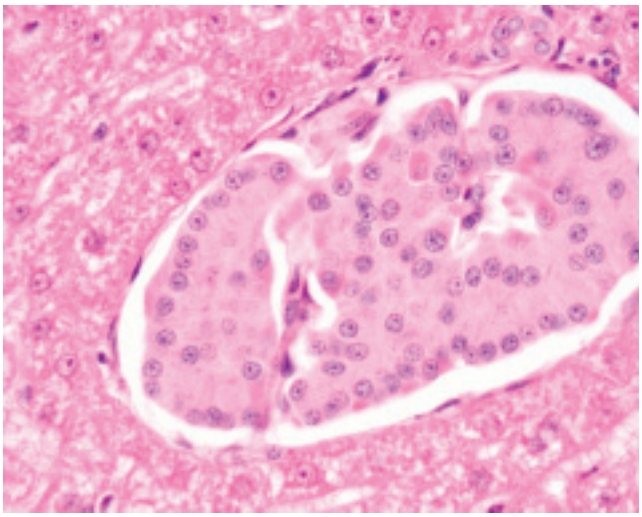
DEVELOPING STRATEGIES TO OVERCOME IMMUNE REJECTION OF TRANSPLANTED CELLS AND ORGANS

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

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

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



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

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

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

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

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

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HIGHLY ACTIVE ANTIRETROVIRAL THERAPY FOR HIV INFECTION—BENEFITS AND DRAWBACKS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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IDENTIFICATION OF A MEDIATOR OF LEPTIN ACTION IN MUSCLE

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

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

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

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

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OF MICE AND MUSCLES: INCREASING STAMINA THROUGH GENE REGULATION

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

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

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

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

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

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

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

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

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

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

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

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

PATIENT PROFILE

Mollie Singer

Living with Type 1 Diabetes

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

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

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

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

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

MOLLIE SINGER

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

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

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

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

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

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

MOLLIE SINGER

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

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

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

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

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

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

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

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

STORY OF DISCOVERY

Leptin—A Potential Treatment for Lipodystrophy

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

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

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

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

STORY OF DISCOVERY

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

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

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

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

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

Diabetes Education at NIDDK: The National Diabetes Education Program

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

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

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

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

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

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

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

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

PATIENT PROFILE

Monica Boone

A Patient in the Diabetes Prevention Program

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

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

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

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

MONICA BOONE

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

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

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

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

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

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

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

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

MONICA BOONE

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

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

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

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

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

PATIENT PROFILE

Aylin Riedel

Management of Diabetes and Kidney Disease as a Daily Balancing Act

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

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

Impact on Life Decisions

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

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

AYLIN RIEDEL

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

The Impact of Research

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

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

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

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

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

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

AYLIN RIEDEL

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

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

Living with the Disease

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

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

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

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

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

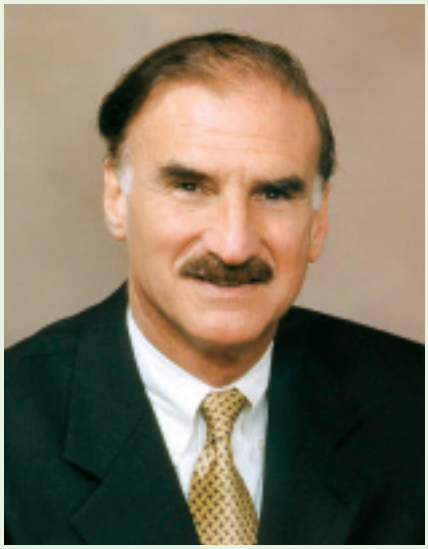
The prevention and treatment of the long-term micro- and macrovascular complications of diabetes—kidney, eye, nerve, and cardiovascular disease—remain critical problems in diabetes care. Because the blood vessel damage leading to these complications can begin as soon as a person becomes diabetic, early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

VISION STATEMENT

C. Ronald Kahn, M.D.

A Vision of the Future: Diabetes Research

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



Dr. C. Ronald Kahn

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

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

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

VISION STATEMENT

Genetics of Diabetes and Its Complications and Obesity

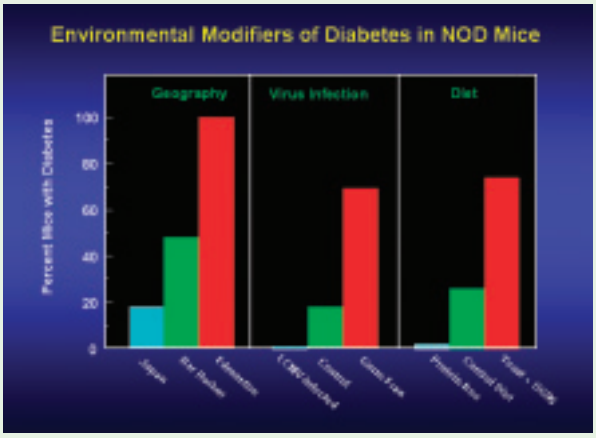
Both type 1 and type 2 diabetes are complex genetic diseases that result from interactions between multiple genes and environmental factors. Approximately 80 percent of the type 2 diabetes in the U.S. occurs in overweight or obese individuals. Real progress has been made in the area of the genetics of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

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

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

VISION STATEMENT

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



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

Autoimmunity and the Beta Cell

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

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

Cell Signaling and Cell Regulation

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

VISION STATEMENT

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

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

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

Clinical Research and Clinical Trials of Critical Importance

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

Challenges for the Future in Diabetes Research

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