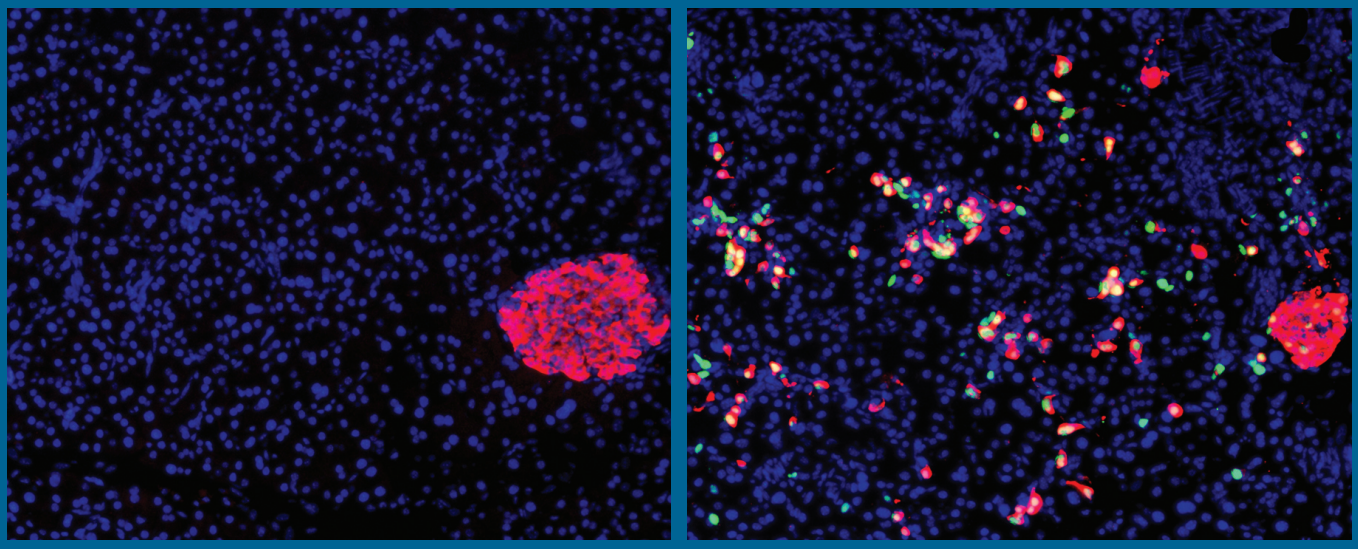

Research highlighted in this chapter describes how adult pancreatic cells can be turned into insulin-producing beta cells. The image on the left shows an adult mouse pancreas, with insulin-producing beta cells (pink) in a little cluster called an islet. Other cells that do not produce insulin are shown in blue. The image on the right shows new beta cells that arise in the adult mouse pancreas after injection of a special, engineered virus containing a specific combination of three factors previously found to be key regulators of pancreatic development. The green color marks many of the cells infected by the virus. Some of the infected cells, although not originally beta cells, turned into insulin-producing cells as a result of the regulatory factors carried by the virus. These are seen as pink cells outside the islet cluster. Other cells are stained blue. Increasing beta cell mass is critically important for both type 1 and type 2 diabetes, and this exciting research identifies a way to generate new beta cells from pre-existing adult cells in a living organism.

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Diabetes, Endocrinology, and Metabolic Diseases

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

Diabetes is a debilitating disease that affects an estimated 23.6 million people in the U.S.—or 7.8 percent of the total population—and is the seventh leading cause of death.[1] Diabetes lowers average life expectancy by up to 15 years,[2] increases cardiovascular disease risk two- to four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult onset blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2007—including costs of medical care, disability, and premature death—was $174 billion.[1] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed.[1]

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

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

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

In patients with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. Gradually, the pancreatic beta cells secrete less and

[1] *http://www.cdc.gov/diabetes/pubs/factsheet07.htm*

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

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

less insulin, and the timing of insulin secretion becomes abnormal. Treatment approaches for controlling glucose levels include diet, exercise, orally administered medications, and, in some cases, injected insulin. There are also an estimated 57 million adults in the U.S. who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[4] This population is at high risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with pre-diabetes can dramatically reduce their risk of developing full-blown diabetes with improvements in lifestyle or with drug treatment.

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

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

GENETICS OF DIABETES

Consortium Identifies Six More Genetic Variants Affecting Likelihood of Type 2 Diabetes: Type 2 diabetes, by far the most common form of diabetes mellitus, is caused by a complex interaction of genes and the environment. Diabetes is much more common in some ethnic groups than in others, suggesting that genes may explain these health disparities. Type 2 diabetes is also strongly associated with obesity, but most of the genetic components identified so far are unrelated to factors influencing obesity. Indeed, most obese people do not develop diabetes, and some people with normal body weight do. Understanding the reasons why one person develops the disease and another does not is a critical research priority. However, until recently, there has been little definitive information about the genetic variants that predispose or protect a person from type 2 diabetes. In the last 2 years, three independent genome-wide association studies have employed powerful new genomic tools to identify 10 common genetic variants, each of which has a modest effect on the probability of a person developing type 2 diabetes.

Because a large sample size is required to uncover genetic variants that have relatively small effects, researchers from the three previous genome-wide association studies formed a consortium to combine their data to potentially identify additional type 2 diabetes genes. They also confirmed their results in samples from several other studies. The larger effective sample size—which combined genetic data from more than 70,000 people—provided the statistical power to identify 6 more common variants associated with an effect on the likelihood of developing type 2 diabetes, raising the known total to 16 genes. In the case of all of the new genes, and most of those previously identified, the precise genetic changes that influence the development of diabetes remain unknown—only their genetic neighborhood has been identified for certain. None of the new genetic variants were previously known to be associated with type 2 diabetes. Interestingly, the new gene that was most strongly associated with risk for type 2 diabetes was previously found also to be associated with prostate cancer.

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

When each genetic region found to influence development of type 2 diabetes has been carefully investigated, the precise genetic changes that exert these effects will be identified. Study of these genes should lead to greater biological understanding of the development of this serious disease, and may lead to new and better diagnostics and personalized treatments. Because the new genetic regions had not previously been associated with type 2 diabetes, this research opens up novel avenues for investigation of underlying causes of the disease. Furthermore, recent analysis of data from the NIDDK's landmark Diabetes Prevention Program clinical trial confirmed that a particular gene variant increases risk for type 2 diabetes in people participating in the trial. However, even the participants at highest genetic risk benefited from healthy lifestyle changes shown to prevent or delay development of type 2 diabetes. This result was very encouraging because, despite a person's genetic risk, lifestyle change could still reduce risk for developing type 2 diabetes.

Zeggini E, Scott LJ, Saxena R, and Voight BF, for the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium: Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40: 638-645, 2008.

Genetic Variation Contributes to the Regulation of Glucose Levels in Humans: Researchers have discovered variations in a region of DNA that are associated with differences in the level of blood glucose (sugar) in the human body. A recent boom of genetic information has enabled scientists to correlate markers in a person's genome—often a difference in a single letter in the DNA sequence—with the likelihood of developing particular diseases or conditions. In previous research, scientists compared the genomes of a large number of individuals—called genome-wide association studies—and identified several regions in the genome that associated with an increased risk for the development of type 2 diabetes. Because elevated glucose levels are associated with increased risk for diabetes, and untreated diabetes leads to a dramatic increase in glucose levels in a person's blood, investigators analyzed this genetic information to determine whether there are correlations between an individual's markers and his or her blood glucose levels. The scientists combined genomic data from two previous studies, the Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) and the SardiNIA Study of Aging, to scan a total of over 5,000 individual genomes. They looked only at people without diabetes to ensure that the measured blood glucose levels had not been artificially lowered by medications that individuals with diabetes may take. They also made statistical adjustments to account for increases in blood glucose associated with elevated body weight, because overweight and obesity are also associated with biologic changes that could increase glucose levels. By correlating genetic data with blood glucose levels measured after overnight fasting, it was determined that multiple markers in the DNA sequence were associated with variation in blood glucose levels. An additional 18,000 genomes of individuals who did not have diabetes were analyzed, using data from seven other studies, to verify the marker with the strongest association.

The marker with strongest association to blood glucose levels is near two different genes, either of which may be responsible for the effect. One of these genes plays an important role in glucose metabolism and thus seems likely to play a role in the regulation of blood glucose levels, while the other has not previously been demonstrated to participate in glucose level regulation. Further research is needed to determine whether and how either of these genes contributes to blood glucose level variation. This study is an important step toward better understanding of blood glucose regulation, and provides new insight into genetic contributions to elevated glucose levels, a precursor to type 2 diabetes. This exciting advance could inform the development of new therapies.

Chen W-M, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orrù M, Grazia Piras M, Bonnycastle LL, Willer CJ, Lyssenko V, Shen H, Kuusisto J, Ebrahim S, Sestu N, Duren WL, Spada MC, Stringham HM, Scott LJ, Olla N, Swift AJ, Najjar S, Mitchell BD, Lawlor DA, Smith GD, Ben-Shlomo Y, Andersen G, Borch-Johnsen K, Jørgensen T, Saramies J, Valle TT, Buchanan TA, Shuldiner AR, Lakatta E, Bergman RN, Uda M, Tuomilehto J, Pedersen O, Cao A, Groop L, Mohlke KL, Laakso M, Schlessinger D, Collins FS, Altshuler D, Abecasis GR, Boehnke M, Scuteri A, and Watanabe RM: Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. J Clin Invest 118: 2620-2628, 2008.

AUTOIMMUNITY IN TYPE 1 DIABETES

Discovery of a New Marker for Pre-clinical Type 1 Diabetes: Scientists have discovered a powerful new tool to help identify people likely to develop type 1 diabetes before symptoms occur. The discovery may facilitate prevention strategies currently in development. A general feature of the autoimmune destruction of insulin-producing pancreatic beta cells that occurs in type 1 diabetes is the body's development of antibodies to beta cell proteins. These antibodies are called autoantibodies because they attack proteins within the body, rather than invading pathogens. Importantly, these autoantibodies generally appear before overt symptoms of diabetes, and are therefore useful as clinical predictors of the disease. The utility of known autoantibodies is limited, however, because some are transient and do not persist until diagnosis, and some people with type 1 diabetes do not develop any of the autoantibodies used in existing screening tests.

To enhance understanding of the pathogenesis of type 1 diabetes and elucidate potential new therapeutic strategies, as well as to improve testing for autoimmunity, a group of researchers sought to identify additional beta cell proteins that generate autoantibodies. By examining a set of proteins made exclusively or almost exclusively in beta cells, and testing with antibodies taken from people with new-onset diabetes, scientists in the NIDDK-funded Beta Cell Biology Consortium (BCBC) have discovered that autoantibodies to a beta cell protein called ZnT8 are an excellent marker for pre-clinical diabetes. Taking advantage of samples collected through the NIDDK's Diabetes Autoimmunity Study in the Young (DAISY), BCBC scientists found that ZnT8 autoantibodies can substantially improve prediction of diabetes when used in combination with the previously discovered autoantibodies commonly used to monitor for pre-clinical type 1 diabetes in research studies. It appears that testing for this combination of autoantibodies can substantially improve the ability to predict risk for developing the disease. Improved risk prediction will facilitate the development, testing, and future administration of therapies to prevent or cure this disease. Once such therapies are available, it is likely that children will be monitored for pre-diabetic autoimmunity and the availability of this test will improve the accuracy of such monitoring. Studies such as DAISY have already shown that identifying individuals with pre-clinical type 1 diabetes can preempt dangerous diabetic episodes in undiagnosed children.

Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, and Hutton JC: The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA 104: 17040-17045, 2007.

New Clues to the Link Between Type 1 Diabetes Susceptibility and the Immune System—Effects of the *PTPN22* Gene: By studying a genetic variant associated with an increase in risk for the development of type 1 diabetes and other autoimmune diseases, scientists discovered how this variant alters the body's immune system. The variant is a change in the DNA sequence of the gene *PTPN22*, which produces a protein that normally inhibits the activation of the T cells of the immune system. The scientists sought to understand how this gene variant could lead to a complicated disease.

In this study, investigators characterized T cells from people who had the *PTPN22* gene variant. To avoid confounding factors that may be associated with a complex disease, they chose volunteers who had not developed an autoimmune disease. T cells, like most cells, have two copies of every gene, and the T cells from these people had one copy with the change and one normal copy of the gene. The scientists found that the cells with the variant did not function normally based on several laboratory tests. For example, they had decreased levels of a signaling protein (IL-10) that T cells produce to affect other immune cells. These results indicated that the variant in *PTPN22* blocked the biologic steps required for activating T cells during an immune response. In addition, these changes were specific to a subset of T cells called "memory" T cells.

To further understand the effects of the *PTPN22* variant within the human body, the scientists looked at the overall composition of the T cell population in people with the variant, and found that they had more memory T cells than people without the variant. When the scientists explored potential effects on another type of immune cell, B cells (the cells that make antibodies), they noticed a reduction in the

number of a subset of B cells called memory B cells. These results suggested that the variant in *PTPN22* led to differences in the populations of both T and B cell types in the human body. The scientists propose that the many changes they observed in immune cells could create an environment that is susceptible to the development of autoimmunity. By uncovering how a particular gene variant that is associated with disease alters the immune system, scientists are gaining a better understanding of the development of autoimmune diseases, like type 1 diabetes, and identifying potential new targets for therapy.

Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, and Buckner JH: Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol 179: 4704-4710, 2007.

Targeting the Immune System To Combat Type 1 Diabetes: Several recent studies suggest that manipulating dendritic cells of the immune system is a promising strategy to prevent, delay, or reverse type 1 diabetes. Type 1 diabetes is an autoimmune disease in which the patient's immune system destroys the insulin-producing beta cells of the pancreas. Central to the attack on the beta cells are immune system cells called T cells. T cells that recognize proteins from the patient's beta cells are normally eliminated during their maturation. However, in susceptible individuals, these T cells migrate to the pancreas and initiate an inflammatory process that destroys the beta cells and leads to the development of type 1 diabetes. Dendritic cells, also immune system cells, are involved in activating T cells. Recent studies have further developed approaches to modify dendritic cells to prompt the elimination of errant T cells to prevent the destructive immune attack.

In one study, scientists modified dendritic cells in culture to have high levels of a signaling protein called IL-4. Previous studies found that both humans and mice with type 1 diabetes have low levels of IL-4. Injection of these modified dendritic cells into a mouse model of type 1 diabetes significantly prevented or delayed onset of disease. By 30 weeks of age, 80 percent of non-treated mice developed the disease, compared to only 30 percent of the mice treated with the modified cells. This study used dendritic cells that were modified outside of the mouse. Other researchers are developing approaches to modify dendritic cells directly in the animal. For example, one study examined whether "microspheres" could be used to deliver molecules known to shut off CD40, CD80, and CD86, which are proteins found on cells of the immune system. These molecules modify dendritic cells in a way to make them suppress, rather than incite, T cell attacks on beta cells. A single injection of microspheres containing these suppressive molecules significantly delayed onset of diabetes in a mouse model of type 1 diabetes; several consecutive injections prevented the disease altogether. In mice that already had diabetes, the microsphere therapy reversed the disease. In another study using mice, researchers used an antibody to a protein found on dendritic cells to deliver specific molecules—ones that mimic a beta cell component and are recognized by diabetes-inducing T cells—to these dendritic cells. This approach led to the elimination of the destructive T cells. Together, these studies demonstrate that modifying dendritic cells is a possible therapeutic approach for preventing, delaying, or reversing type 1 diabetes. Additional studies to test dendritic cell therapy are needed to determine if it might have the same dramatic benefits in people.

Creusot RJ, Yaghoubi SS, Kodama K, Dang DN, Dang VH, Breckpot K, Thielemans K, Gambhir SS, and Fathman CG: Tissue-targeted therapy of autoimmune diabetes using dendritic cells transduced to express IL-4 in NOD mice. Clin Immunol 127: 176-187, 2008.

Mukhopadhaya A, Hanafusa T, Jarchum I, Chen Y-G, Iwai Y, Serreze DV, Steinman RM, Tarbell KV, and DiLorenzo TP: Selective delivery of beta cell antigen to dendritic cells in vivo leads to deletion and tolerance of autoreactive $CD8^+$ T cells in NOD mice. Proc Natl Acad Sci USA 105: 6374-6379, 2008.

Phillips B, Nylander K, Harnaha J, Machen J, Lakomy R, Styche A, Gillis K, Brown L, Lafreniere D, Gallo M, Knox J, Hogeland K, Trucco M, and Giannoukakis N: A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. Diabetes 57: 1544-1555, 2008.

GUT BACTERIA AND TYPE 1 DIABETES

Gut Microbes Protect Against Type 1 Diabetes in Mice: Research in mice has found that the trillions of bacteria and other microbes that live in the gut can blunt the immune system attack that causes type 1

diabetes. The discovery may shed light on rising rates of type 1 diabetes in developed countries. Scientists don't know exactly what triggers the body's immune attack on beta cells in type 1 diabetes. During the past decades, researchers saw clues in the observed increased incidence of type 1 diabetes in developed countries. The scientists suspected that changes in the environment, including the microbes that live in our bodies, may be influencing the disease. Supporting this idea, previous studies found that the incidence of type 1 diabetes in mice susceptible to this disease can be affected by microbes in their environment. The researchers set out to further explore the possible connection between type 1 diabetes and microbes.

Receptors on certain immune cells recognize molecular patterns that mark the surface of microbes. These immune cells signal through a protein called MyD88 to launch an immune system response. When researchers disrupted the gene for MyD88 in a mouse model of type 1 diabetes, the mice no longer developed the disease. While the researchers confirmed that immune activation in the MyD88-deficient mice was suppressed in pancreatic lymph nodes, it was not eliminated. Thus, type 1 diabetes prevention was likely more than simply a matter of turning off part of the immune system. The researchers therefore raised the mice in a germ-free environment. These same mice developed type 1 diabetes when raised in this type of environment, showing that the disease is not dependent solely on the MyD88 pathway. The researchers next gave the germ-free mice a defined mix of "friendly" gut bacteria and found that the incidence of diabetes was significantly reduced. These experiments show that a complex interaction between the immune system and bacteria in the gut may help to lower the risk of developing type 1 diabetes. The widespread use of antibiotics and more aggressive cleanliness of modern society can alter the mix of microbes living in our body. This research suggests that an unintended consequence of this environmental change is an increased risk of autoimmune diseases like type 1 diabetes. The idea opens avenues for further exploration and hints at the possibility of developing bacteria-based treatments for people with autoimmune diseases.

Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, and Chervonsky AV: Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature 455: 1109-1113, 2008.

Reprinted, in a slightly modified form, from NIH Research Matters*; original article by Harrison Wein, Ph.D., published on September 29, 2008.*

BETA CELLS AND DIABETES

Adult Pancreas Cells Reprogrammed to Insulin-producing Beta Cells: New research has taken a step closer to cell replacement therapy for diabetes. Scientists made an exciting discovery that some adult cells in the mouse pancreas, called exocrine cells, can be reprogrammed to become insulin-producing beta cells. Beta cells are at the center of the development of both type 1 and type 2 diabetes, and researchers are vigorously trying to find ways to replace damaged or destroyed beta cells in people with diabetes.

One way to approach this difficult task is to reprogram different adult cell types into beta cells. The pancreas is made up of many different cell types, of which exocrine cells are the most plentiful. To identify factors to reprogram exocrine cells, they focused on proteins called transcription factors, which regulate whether genes are turned on or off. Although over 1,100 transcription factors are known to be important in pancreatic development, the scientists limited their experiments to testing nine transcription factors of key importance, based on knowledge from earlier research on pancreatic development from embryonic to adult stages.

Using a genetically-engineered virus, they delivered a mix of the nine factors into pancreases of mice. By removing one factor at a time from the mix, the researchers identified a combination of just three transcription factors that reprogrammed some of the exocrine cells into beta cells. The newly-formed beta cells produced enough insulin to decrease high blood glucose levels in diabetic mice. This "reprogramming" appeared to occur directly from the exocrine cell type to the beta cell type, and the procedure did not require the addition of stem cells. If the same type of approach works in humans, this discovery could have a dramatic impact on the ability to increase beta cell mass in people with diabetes. While much work remains to be done before this becomes a safe and effective therapy, this adult cell reprogramming is a major step forward and serves as a model for other applications of regenerative medicine.

Zhou Q, Brown J, Kanarek A, Rajagopal J, and Melton DA: In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455: 627-632, 2008.

New Insights into the Molecular Contributors to Gestational Diabetes Mellitus: Researchers have identified a protein that may be involved in the development of gestational diabetes mellitus (GDM). GDM is a form of glucose intolerance diagnosed in some women during pregnancy. Pregnancy is a time when the body becomes slightly less sensitive to insulin and more insulin is needed to compensate. Studies suggest that, to meet this demand, insulin-producing beta cells proliferate. However, the molecular mechanisms underlying pregnancy-induced beta cell proliferation are poorly understood. Furthermore, it is unclear if impaired beta cell proliferation plays a role in the development of GDM.

To enhance understanding of the molecular underpinnings of beta cell proliferation during pregnancy, researchers studied a protein called menin. This protein had previously been found to play a role in cancer. Loss of menin promotes neuroendocrine tumors, including beta cell tumors. Because these observations suggest that this protein may regulate the growth of beta cells, researchers hypothesized that it may play a role in beta cell growth during pregnancy. They discovered that levels of menin, as well as levels of proteins controlled by menin, decreased during pregnancy in mouse islets, and then returned to prepartum levels after birth, corresponding to observed changes in maternal beta cell mass. To determine the effect of artificially increasing menin levels during pregnancy, the researchers generated a genetically-engineered mouse model in which they could induce higher levels of the protein in the animals' beta cells. They observed that increased menin levels during pregnancy caused the mice to have features of human GDM, including high blood glucose and insufficient insulin levels. In addition, pregnancy-induced beta cell proliferation was inhibited in these animals. The researchers also observed that administration of prolactin, a hormonal regulator of pregnancy, to non-pregnant mice reduced menin levels in islets and increased beta cell proliferation. These results suggest that menin is a key regulator of beta cell proliferation during pregnancy.

This study in mice sheds new light on the molecular mechanisms that underlie beta cell proliferation during pregnancy and the development of GDM. Menin was discovered by investigators in the NIDDK Intramural Research Program as the gene defective in a rare endocrine disorder. Further understanding of the role of this protein sheds light on GDM and mechanisms of beta cell proliferation. If it plays a similar role in regulating beta cell proliferation in humans, then menin—or other proteins in the menin pathway—may be potential therapeutic targets for treating or preempting GDM and other forms of diabetes.

Karnik SK, Chen H, McLean GW, Heit JJ, Gu X, Zhang AY, Fontaine M, Yen MH, and Kim SK: Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. Science 318: 806-809, 2007.

DIABETES AND ITS COMPLICATIONS

First Year of Look AHEAD (Action for Health in Diabetes) Trial Yields Encouraging Results: The Look AHEAD trial has enrolled over 5,000 participants to determine the effects of an intensive lifestyle intervention on the long-term health of overweight and obese adults with type 2 diabetes. At enrollment, the study population had an average age of 59 years with 60 percent female and 37 percent minority. In addition, approximately 85 percent were obese and 87 percent were on diabetes medications. Few individuals were achieving optimal control of hemoglobin A1c (HbA1c, a measure reflective of blood glucose control over the preceding 2-3 months), blood pressure, and "bad" or low density lipoprotein (LDL)-cholesterol levels. High blood glucose, blood pressure, and cholesterol may all contribute to diabetes-related vascular complications, including cardiovascular, eye, and kidney disease; therefore, control of these three conditions is especially important in people with diabetes. At enrollment, only 10 percent of individuals in the Look AHEAD population were meeting all three goals established by the American Diabetes Association (ADA) of HbA1c less than 7.0 percent, blood pressure less than 130/80 mmHg, and LDL-cholesterol less than 100 mg/dl. Among Caucasians, 11.6 percent met all three goals, while only 5.1 percent of African Americans and 7.9 percent of Hispanics did. Examples of other

factors associated with suboptimal control of all three measurements included higher body mass index (BMI, a measure of weight relative to height), lower level of education, and longer duration of diabetes. Individuals who used diabetes medication also were less likely to meet all three goals than individuals who were not on diabetes medication. This seemingly contradictory result may be due, in part, to medication being prescribed to those individuals with longer duration of diabetes whose disease was at a more advanced stage and thus more difficult to control.

As reported in the 2008 edition of *NIDDK's Recent Advances & Emerging Opportunities*, first year results of the trial showed that overweight or obese people with diabetes who had received a year of intensive lifestyle intervention—including regular individual and group counseling, structured meal plans, and customized exercise programs—lost an average 8.6 percent of their initial body weight. By comparison, a similar group that received standard diabetes education and support lost only 0.7 percent of their body weight. This 7 to 10 percent weight loss had a big clinical impact. HbA1c, blood pressure, and LDL-cholesterol improved in both groups, but participants in the lifestyle intervention group saw greater reductions. In the lifestyle group, the percent of people meeting all three ADA goals more than doubled. From a beginning average of 7.3 percent, the mean HbA1c level dropped to 6.6 percent in the lifestyle intervention group, versus 7.2 percent in the group that received the usual care. Lifestyle intervention group participants also reduced their use of diabetes, blood pressure, and lipid-lowering medicines, while improving control of these factors.

Bertoni AG, Clark JM, Feeney P, Yanovski SZ, Bantle J, Montgomery B, Safford MM, Herman WH, and Haffner S; The Look AHEAD Research Group: Suboptimal control of glycemia, blood pressure, and LDL cholesterol in overweight adults with diabetes: the Look AHEAD Study: J Diabetes Complications 22: 1-9, 2008.

The Look AHEAD Research Group: Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: One-year results of the Look AHEAD trial: Diabetes Care 30: 1374-1383, 2007.

Hearing Loss Is Common in People with Diabetes: A recent study has found that hearing loss is about twice as common in adults with diabetes compared to those who do not have the disease. NIH scientists analyzed data from hearing tests administered from 1999 to 2004 to participants in the National Health and Nutrition Examination Survey, which is conducted by the Centers for Disease Control and Prevention (CDC). Over 5,100 survey participants aged 20 to 69 completed a hearing exam that used a machine to measure hearing sensitivity across a range of sound frequencies—low, middle, and high frequency. These participants also completed a questionnaire in which they were asked to report if they had ever been told by a doctor that they had diabetes. After accounting for differences in age, the prevalence of low- or mid-frequency hearing impairment of mild or greater severity in the worse ear was about 21 percent among 399 adults with diabetes, compared to about 9 percent among 4,741 adults without diabetes. For high frequency sounds, mild or greater hearing impairment in the worse ear was found in 54 percent of those with diabetes, compared to 32 percent of those who did not have the disease. Greater hearing impairment in people with diabetes was evident as early as ages 30 to 40. The association between diabetes and hearing loss was observed even after accounting for other factors (in addition to age) that are known to affect hearing, including race, ethnicity, income level, noise exposure, smoking, and the use of certain medications. The researchers also evaluated a subset of participants and found that adults with pre-diabetes had a 30 percent higher rate of hearing loss compared to those with normal blood glucose tested after an overnight fast. Although the study did not directly address how diabetes may cause hearing loss, evidence from other studies suggests that the nerve and blood vessel damage that occurs in diabetes and leads to eye, kidney, and nerve complications may also affect the inner ear. This study of a large, nationally representative population sample indicates that hearing loss may be an under-recognized complication of diabetes that health care providers should be aware of in managing the care of people with diabetes.

Bainbridge KE, Hoffman HJ, and Cowie CC: Diabetes and hearing impairment in the United States: Audiometric evidence from the National Health and Nutrition Examination Survey, 1999 to 2004. Ann of Int Med 149: 1-10, 2008.

Depression and Diabetes—Clues to the Relationship Between Physical and Mental Health: Scientists have determined that depression and type 2 diabetes

can influence one another, and are making strides in understanding the biology behind this relationship. In previous studies, depression and depressive symptoms were observed to be more common among people with diabetes than in the general population. But does a diagnosis of diabetes increase the risk of developing depression? Does depression increase the risk of developing diabetes? In one study to understand the relationship between these two complex conditions, scientists analyzed the mental and the physical health of men and women enrolled in the National Heart, Lung, and Blood Institute's Multi-Ethnic Study of Atherosclerosis. Each volunteer participated in multiple examinations over an average of 3 years, including evaluations of their blood glucose levels, development and/or progression of diabetes, and quality of life to assess their mental well-being. Since many variables are likely to affect both diabetes and depression, the scientists also documented factors like age, ethnicity, gender, body mass index, socioeconomic factors, lifestyle, and measures of metabolic and inflammatory markers.

The investigators examined the data to determine the frequency of development of diabetes among participants who already had symptoms of depression, and the development of depressive symptoms among participants diagnosed with diabetes. The scientists noted that people with multiple depressive symptoms had a modestly increased risk of developing type 2 diabetes. These results suggest that it may be important to consider future studies to determine whether interventions that treat depression may be of benefit in preventing type 2 diabetes. In addition, the investigators observed that individuals being treated for type 2 diabetes had a significantly greater risk of developing depressive symptoms. These results suggest that physicians treating people with type 2 diabetes should consider routinely screening their patients for depressive symptoms.

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Intensive Lowering of Blood Glucose May Be Harmful to Patients with Type 2 Diabetes and a High Risk of Cardiovascular Disease: A large clinical trial has shown that, for individuals with type 2 diabetes who were also at especially high risk of heart disease and stroke, the use of intensive therapy to lower blood glucose (sugar) levels to near normal levels led to increased mortality and did not significantly reduce major cardiovascular events.

Type 2 diabetes is characterized by high blood glucose levels, and having diabetes increases the risk of many serious health complications, including cardiovascular disease. In addition, people with type 2 diabetes may be overweight and have blood pressure and lipid or cholesterol levels that further add to their cardiovascular disease risk. Prior studies suggested that reducing blood glucose levels to those found in adults without diabetes may reduce the risk of cardiovascular events, such as heart attack and stroke, among people with type 2 diabetes. However, a randomized clinical trial was needed to determine the validity of this hypothesis.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial, led by the National Heart, Lung, and Blood Institute with support from the NIDDK, enrolled over 10,000 adult volunteers who had type 2 diabetes and an especially high risk of cardiovascular disease. The trial was designed to test three treatment approaches to decrease the high rate of major cardiovascular events observed in people with this combination of health problems. The treatment approaches included intensive lowering of blood glucose levels, intensive lowering of blood pressure, and treatment of blood lipids (i.e., fats like cholesterol). Each of the intensive therapies was compared with a more standard therapy. While the study to test the effect of intensively lowering blood glucose levels has ended, the two other studies are currently ongoing.

Blood glucose levels are measured by a test called hemoglobin A1c (HbA1c), which reflects a person's average level of blood glucose in the previous 2 to 3 months. The ACCORD trial randomly assigned participants, who had on entry an average HbA1c of 8.1 percent, to either intensive therapy to lower HbA1c to a target of below 6.0 percent—a level close to normal for adults without diabetes—or to standard therapy to achieve an HbA1c target of 7.0-7.9 percent. ACCORD participants received medications to lower their blood glucose levels; the type, number, and dosages of the drugs varied, depending on the participants' individual needs and the HbA1c goal.

Participants in the intensive therapy group achieved, on average, HbA1c values lower than standard therapy group participants; half achieved an HbA1c of less than 6.4 percent after 1 year. After following volunteers for an average of 3.5 years, however, the researchers observed that intensively lowering blood glucose levels actually increased mortality in this group. Moreover, it did not significantly reduce major cardiovascular events compared to standard therapy. Although patients in both the intensive and standard groups had lower mortality than reported in other studies of similar patients, the NIH, on the advice of a committee monitoring the study, decided to end this part of the study, and all participants were switched to treatment with standard therapy.

Participants in the ACCORD trial had already had diabetes for an average of 10 years and were either at particularly high risk for a cardiovascular event (two or more risk factors beyond diabetes) or had been diagnosed with cardiovascular disease before entering the study. Thus, the results of this study may not apply to patients with type 2 diabetes who are not similar to the ACCORD participants. After the results of this study were released, diabetes experts agreed that the blood glucose level goal currently recommended by the American Diabetes Association (an HbA1c level of 7 percent) continues to be appropriate for the general population with diabetes. However, less aggressive therapy may be appropriate for individuals with limited life expectancy, cardiovascular disease, or frequent low blood glucose reactions. Importantly, people with diabetes should talk with their doctor before making any changes to their treatment regimen.

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REGULATORS OF METABOLISM IN HEALTH AND DISEASE

A Novel Pathway To Promote New Blood Vessel Growth: Scientists have discovered a novel molecular pathway in mice that promotes new blood vessel growth, a process called angiogenesis. Angiogenesis is critically important to the development and growth of a healthy embryo. It is also important for health in adults; numerous diseases are caused by either excessive or insufficient angiogenesis. Research previously showed that low levels of oxygen in the body are detected by a group of proteins called "hypoxia inducible factors." These proteins activate the production of vascular endothelial growth factor, or VEGF, which stimulates angiogenesis so that the body receives sufficient supplies of oxygen and nutrients from the blood. Researchers have now demonstrated that another protein, called PGC-1alpha, is also a key regulator of angiogenesis. The scientists first showed that, in cell culture, levels of this protein increase when the cells are exposed to low levels of oxygen or nutrients. Increased levels of PGC-1alpha in turn lead to increased levels of VEGF and other factors known to be involved in promoting angiogenesis. Then, to determine if PGC-1alpha regulates angiogenesis in an animal model, the researchers studied two different groups of genetically-engineered mice: one group produced high levels of PGC-1alpha in their skeletal muscle and the other group lacked the protein altogether. The researchers observed that angiogenesis was accelerated in the mice that had high levels of the protein, whereas angiogenesis was impaired after injury in the mice that lacked it. These results suggest that PGC-1alpha is an important regulator of angiogenesis in a mouse model. Surprisingly, the protein does not stimulate angiogenesis through the well-known signaling pathway involving the hypoxia inducible factors. Rather, it uses an alternate route—a novel signaling pathway that involves a protein called ERR-alpha, which regulates whether certain genes are turned on or off. The two proteins work together to increase levels of VEGF, which in turn promotes angiogenesis.

Decreases in blood supply to the heart, brain, and limbs are a leading cause of morbidity and mortality throughout the world, and scientists are actively trying to develop therapies to target angiogenesis. The discovery of a second pathway involved in new blood vessel growth, which involves PGC-1alpha and ERR-alpha, may result in new opportunities for therapy. PGC-1alpha has already been well studied for its role in diabetes and obesity, and molecules that increase its activity are in development.

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Powering Up Mice with PEPCK-C: A fascinating new discovery in energy metabolism may point the way to new therapeutic approaches for obesity. In humans and other mammals, the use and storage of energy—or energy metabolism—is driven by millions of chemical reactions taking place within cells. Some reactions release energy to power daily activities, for example, while others transform it into fat and other energy storage molecules. Now, scientists have found that, in mice, changing the amount of a single protein in just one tissue leads to a profound change in energy metabolism. The protein, an enzyme called PEPCK-C, helps some body tissues make glucose (sugar) and related energy-rich molecules for immediate use or storage. While it plays a key role in the functions of liver, kidney, and fat tissue, the PEPCK-C enzyme is not normally present in high amounts in skeletal muscle, a major site of energy metabolism. In a recent study, researchers used genetic engineering to create mice that overexpress the PEPCK-C gene in their skeletal muscle. These "PEPCK-C mice" make about 100-fold more active enzyme in their skeletal muscle than normal. When compared to "control" mice that don't have extra PEPCK-C, the PEPCK-C mice were not only much more active in their cages, but they also were able to run up to 30 times farther on a treadmill. Moreover, although they ate about 60 percent more food, the highly active adult PEPCK-C mice weighed only half as much as the control mice and had only one-tenth the body fat. When the researchers examined skeletal muscle cells from the PEPCK-C mice, they saw that the cells had many more mitochondria—the so-called powerhouses of the cell—which control much of cellular energy metabolism. The mice also had significantly more intramuscular fat, which the mitochondria can convert to energy—possibly explaining in part how these mice could fuel their high activity. Finally, despite their higher metabolism, the PEPCK-C mice were actually reproductively active longer and lived longer on average than the control mice. Strikingly, the results of PEPCK-C overexpression in skeletal muscle observed in this study stand in sharp contrast to the effect of higher-than-normal levels of the same enzyme in mouse fat tissue, which were found in earlier studies to cause obesity. The mechanisms underlying these intriguing and contrasting results in mice have yet to be fully explained. However, the study provides new opportunities for exploration to understand energy metabolism and, perhaps eventually, to reduce the onset or effects of overweight and obesity.

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A New Chemical Tool for Studying a Protein Implicated in Type 2 Diabetes: Following synthesis in the cell, proteins are often modified by addition of other chemicals that give them important, new biochemical properties. One common type of protein modification is glycosylation—the addition of sugars. A recent NIDDK intramural study sought to provide tools for better understanding an enzyme that catalyzes the reverse process—deglycosylation—for certain sugars on certain proteins. A mutation in the gene that encodes the enzyme, *O*-GlcNAcase, has been implicated in type 2 diabetes in a Mexican American population. *O*-GlcNAcase is found in two forms in the cell, one long and one short. Although the long form of the enzyme is easily studied using previously described biochemical inhibitors, scientists lacked an inhibitor that is effective against the short form. This new study describes an inhibitor that is significantly more potent against the short form of *O*-GlcNAcase, providing a useful tool for studying this important enzyme, and potentially for understanding the role of the enzyme in type 2 diabetes.

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CYSTIC FIBROSIS RESEARCH

New Animal Model To Study Cystic Fibrosis: Scientists have generated a new animal model to advance research on cystic fibrosis (CF). Animals are important in research because they can be used to study underlying mechanisms of disease and to test

new therapies before they are tested in people. Animal models are most useful if they closely mimic the human disease. This has not been the case for CF, because mice with the human CF mutation, for example, do not have the same characteristics of human disease, thus limiting their usefulness in CF research. Scientists sought to generate an animal model that more closely resembles human CF. They chose pigs, because pig and human anatomy and physiology are comparatively similar. The scientists genetically engineered pigs to lack the gene that is mutated in CF, called *CFTR*. The newborn piglets lacking *CFTR* had remarkably similar characteristics to newborn humans with CF, including abnormalities involving the pancreas, intestine, gallbladder, and liver. Lung disease in CF is caused by infection and inflammation. Which comes first remains an important question. At birth, researchers found no evidence of infection or inflammation in the pigs, a situation similar to newborn humans with CF. Scientists can use the pig model to gain a greater understanding of how lung disease develops in CF. Because the new pig model closely mimics the human disease, it is an important new resource for scientists studying CF. The pig model provides new opportunities to enhance understanding about CF and to develop new treatment strategies.

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STORY OF DISCOVERY

Ability To Predict Type 1 Diabetes Offers Hope for Disease Prevention

Type 1 diabetes is a devastating disease that most often strikes during childhood, and invariably lasts for the rest of one's life. During every day of the lives of the millions of people with this disease worldwide, consistent attention and vigilance is required to ward off devastating diabetic complications that shorten and reduce the quality of their lives. Therefore, a key goal of NIDDK research is to develop ways to prevent type 1 diabetes from occurring in the first place. Toward realizing that goal, scientists have cleared a critical hurdle by learning how to identify people who are likely to develop the disease.

Being able to predict who will get type 1 diabetes is of obvious importance in identifying people who would benefit from prevention strategies once they are developed. But in fact it is also a key step in the development of interventions to prevent the disease. With the ability to predict type 1 diabetes risk, it becomes feasible to conduct multiple trials in those at risk, so as to increase the possibility of finding the best prevention approach. This is precisely what is being accomplished today through programs like Type 1 Diabetes TrialNet, led by NIDDK, and TRIGR (Trial to Reduce IDDM in the Genetically at Risk), led by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). Both programs are supported in part by the Special Statutory Funding Program for Type 1 Diabetes Research.

The scientific achievement of predicting type 1 diabetes was developed through decades of efforts by scientists in several disciplines—immunology, genetics, and epidemiology—working in several countries. Although the clinical appearance of type 1 diabetes is often sudden, with symptoms developing over weeks or days, researchers now know that the disease frequently develops gradually and silently over many years. A key advance was the ability to detect the autoimmune hallmarks of the disease prior to the actual development of type 1 diabetes. Researchers in the 1960s recognized that people with diabetes often make antibodies to insulin, a hormone produced by pancreatic beta cells that are aberrantly destroyed in type 1 diabetes, necessitating treatment with exogenously-supplied insulin. Because these antibodies often arose prior to insulin treatment, the scientists correctly surmised that the people were actually developing antibodies to the insulin being made by their own bodies. Antibodies against one's own proteins are termed "autoantibodies," and are a hallmark of autoimmune diseases like type 1 diabetes.

Indeed, researchers later discovered that people with type 1 diabetes often produce antibodies not only to insulin, but also to several other proteins produced by pancreatic beta cells. Significantly, the appearance of autoantibodies nearly always precedes the onset of overt symptoms of type 1 diabetes, when a person still has an adequate number of insulin-producing beta cells to control blood glucose. Testing for the presence of beta cell autoantibodies therefore became a promising approach to predicting the disease before its clinical appearance.

Several scientists, including NIDDK-supported researchers, worked to turn the discovery of autoantibodies into a useful tool by developing robust, standardized autoantibody tests. Importantly, they recognized that the simple presence or absence of an autoantibody does not provide as much information as accurate measurement of the levels or titer of antibody in the blood. Assays to measure antibodies can now be performed such that each test has a very low false-positive rate. Although onset of the disease

STORY OF DISCOVERY

is usually preceded by creation of antibodies to at least one of these proteins, at any given time a person destined to develop type 1 diabetes only makes autoantibodies to a variable subset of them. The presence of any one of these autoantibodies signals substantially elevated risk, and risk increases as the number of autoantibodies rises.

But type 1 diabetes is such a complex disease that, to be accurately interpreted, an autoantibody test needs to be viewed in the context of more information about the patient. It has long been known that people with a parent, brother, or sister with the disease are more likely to get type 1 diabetes than the population at large. However, most people with such relations will not get the disease, and many people without a known relative who has the disease will. The reasons for this are complex, but an important part of the answer stems from the fact that several genes turn out to predispose a person to type 1 diabetes, while several others actually have a protective effect.

The first major breakthrough in the genetic part of the puzzle came in the 1970s, with the discovery that two particular versions of a gene called *HLA*, which makes a key immune recognition protein, are much more common in people who have type 1 diabetes, suggesting they increase the likelihood of the disease. It was later discovered that certain other versions of this highly variable gene can help protect against the disease. Still other versions of HLA are more neutral in their impact. A person can acquire a high-risk version from one parent, and a protecting version from the other. The overall effect of the HLA variants accounts for a very large proportion of the genetic risk for type 1 diabetes.

With the knowledge of HLA and autoantibody associations with type 1 diabetes, NIDDK-supported scientists designed a prevention trial, the NIDDK-supported Diabetes Prevention Trial Type 1 (DPT-1), which successfully used genetic and autoantibody tests to predict risk for developing type 1 diabetes. To identify "those at risk," the researchers first selected thousands of people who have a close relative with type 1 diabetes, and then screened them for autoantibodies. Those with autoantibodies were then tested for the protective version of HLA. People who had autoantibodies and no protective HLA were tested for their response to glucose, to see whether they were already displaying signs of diabetes. Indeed, some already had the disease, and simply did not know it. The rest fell into two categories: those with a normal response to a glucose challenge were considered to have a "moderate" (26-50 percent) chance of developing type 1 diabetes within 5 years; those with a response to glucose that was weaker (but did not meet the definition of overt diabetes) were considered to have a greater than 50 percent chance of developing the disease within 5 years. Although the specific prevention strategies tested in this trial did not turn out to have a broadly protective effect, the researchers' estimates of risk for type 1 diabetes, based on their screening, proved to be remarkably accurate. Thus, the DPT-1 trial was enormously valuable in demonstrating that it is possible to identify those at high risk for type 1 diabetes—enabling researchers to conduct further studies to test new prevention strategies.

NIDDK-supported scientists are continuing to discover potential new ways to improve prediction of diabetes risk. For example, researchers recently identified another autoantibody that, when combined with tests for the previously-known autoantibodies, improves the predictive power of this approach. (Please see the advance on a new autoantibody for type 1 diabetes described earlier in this chapter.) Researchers are continuing to discover other genes that impact the probability of developing type 1 diabetes. At current count there are over 40 such genes, largely discovered through the efforts of the Type 1 Diabetes Genetics Consortium, made possible by support from NIDDK and the Special Statutory Funding Program for Type 1 Diabetes Research. Individually, none of these new genes has as large

an impact as HLA, but collectively their effect is significant.

With further genetic, autoantibody, or other predictive markers and tools, it may be possible to define risk for type 1 diabetes even more precisely, and to extend such predictive tests to the population as a whole. Such predictive markers may also help scientists identify potential environmental triggers of the disease. Improved tests to assess risk not only would facilitate additional research on prevention strategies, but could also advance research on ways to reverse the disease in its earliest stages and, importantly, enable the resulting interventions to benefit more people.

STORY OF DISCOVERY

Diabetes and Cardiovascular Disease

Seminal clinical trials have revealed the power of good control of blood glucose (sugar) early during the course of type 1 and type 2 diabetes to reduce later risk for eye, kidney, and nerve complications. Now, clinical trials are examining the more complex relationship between blood glucose control and cardiovascular disease (CVD) in type 2 diabetes. One recent study showed that more intensive control than currently recommended, targeting near normal blood glucose levels, can be dangerous in those with long-duration type 2 diabetes with established CVD or at high risk of developing CVD. Two other recent trials found neither cardiovascular harm nor benefit of moving from "good" to near-normal glucose levels. However, another study found that targeting good glucose control early in the course of disease can reduce cardiovascular risks decades later for many patients with type 2 diabetes. Similar cardiovascular benefits emerging long after a finite period of intensive glucose control were reported previously for individuals with type 1 diabetes. Because CVD is the leading cause of death in people with type 2 diabetes, identification of ways to reduce this risk is particularly important. There is very strong evidence that blood pressure and cholesterol control can markedly reduce CVD, but the effects of glucose control on CVD in type 2 diabetes remained an open question. Taken together, the new results refine the approach to treating diabetes and demonstrate the importance of tailoring therapy to individual patient characteristics.

Diabetes Increases the Risk of Death from Cardiovascular Disease

An estimated 23.6 million Americans have diabetes, about 5.7 million of whom have not been diagnosed.[1] Type 1 diabetes, which accounts for 5-10 percent of diagnosed diabetes cases, is an autoimmune disease that often begins in childhood or early adulthood, although it can strike at any age. The majority of people with diabetes have type 2 diabetes—a form

Drug Therapies for Type 2 Diabetes

There are many medications available to help people with type 2 diabetes lower their blood glucose. These medications fall into several classes:

- Insulin: moves glucose from blood into cells
- Metformin: reduces output of glucose from the liver and reduces insulin resistance
- Thiazolidinediones: reduce insulin resistance, by a different mechanism than metformin
- Sulfonylureas: promote release of insulin by the pancreas
- Meglitinides: promote release of insulin by the pancreas (shorter and faster acting than sulfonylureas)
- D-phenylalanine derivative: promotes release of insulin
- GLP1-analogs: stimulate production of insulin and slow gastric (stomach) emptying
- DPP-4 inhibitors: slow destruction of GLP1 and stimulate production of insulin
- Amylin analogs: slow glucose absorption from intestine, reduce glucose production by liver, and decrease appetite
- Alpha-glucosidase inhibitors: interfere with digestion and utilization of carbohydrates like starch and table sugar

Other promising therapeutic approaches are currently in development.

of the disease that is typically associated with excess body weight and older age. In part due to the increase in childhood obesity, however, children increasingly are being diagnosed with type 2 diabetes. Both type 1 and type 2 diabetes are also influenced by genetic susceptibility. While both forms of diabetes are characterized by excessively high levels of glucose in the blood, type 1 diabetes and type 2 diabetes have different causes and are treated differently, particularly at disease onset. From the moment of diagnosis, because their insulin-producing cells have been destroyed, people with type 1 diabetes must depend on exogenous insulin, provided by injections or an insulin pump, for survival. Type 2 diabetes, in contrast, is often managed with changes in diet and exercise in its early stages. Insulin-producing cells may still be functioning in type 2 diabetes, but not sufficiently to overcome the insulin resistance that characterizes this form of the disease. A wide variety of prescription medications have been developed to help lower blood glucose in people with type 2 diabetes. (See inset box). Because these drugs act in various ways to lower blood glucose levels, some may be used in combination with others. Many people with type 2 diabetes also need to take insulin to optimally control their blood glucose levels, especially after having the disease for many years.

Despite markedly different causes and treatment options, type 1 diabetes and type 2 diabetes share a common outcome: excess glucose in the blood gradually leads to damaged blood vessels in organs throughout the body. Injury to small blood vessels, known as microvascular disease, increases the risk of blindness, kidney failure, nerve damage, and lower limb amputation. Injury to larger blood vessels, known as macrovascular disease, leads to elevated rates of heart attack, stroke, and other cardiovascular complications in people with diabetes. In general, two out of three adults with diabetes will die of cardiovascular disease or stroke—a risk that is two to four times higher than that for people without diabetes.[1] For people with type 1 diabetes, the risk of death from CVD may be as much as 10-fold greater than the general population of the same age.[2,3] This elevated risk of cardiovascular death shortens the expected life span of people with diabetes by several years.

Long-Term Benefits of Intensive Glucose Control Established for Microvascular Complications

Diabetic complications result from many years of gradual glucose-mediated damage to blood vessels. Thus, clinical trials of new therapies for preventing complications are designed to follow participants' health outcomes over long periods of time following initial treatment.

In 1983, the NIDDK's Diabetes Control and Complications Trial (DCCT) was launched with 1,441 volunteers with type 1 diabetes randomly divided into two groups. One group received what was standard insulin therapy at the time—one or two insulin injections per day. The other group was taught to manage their blood glucose intensively with frequent monitoring of glucose levels and multiple insulin injections daily or use of an insulin pump. The study was designed to test the ability of intensive glucose control to reduce eye damage and other microvascular complications. The study relied on a blood test (HbA1c) which gauges the average blood glucose over the previous 2 to 3 months. A normal HbA1c is below 6 percent. Throughout the study the average HbA1c value in the standard therapy group was 9.1 percent, whereas in the intensive therapy group the value was 7.3 percent—a significant difference in glucose control.

This difference, when maintained over an average of 6.5 years, yielded multiple health benefits: participants in the intensive therapy group exhibited lower rates of eye disease (76 percent reduction in risk), kidney disease (50 percent reduction), and nerve damage (60 percent reduction). Thus, the intervention to improve glucose control was clearly an effective means to lower the risk of microvascular complications in type 1 diabetes. However, because the DCCT participants were relatively young and healthy at

the start of the trial, and because CVD typically takes a longer time to develop than other diabetes complications in patients with type 1 diabetes, it was not possible for researchers to assess the effect of intensive glucose control on cardiovascular risks during the 10 years of the trial.

Longer follow-up demonstrated additional benefits. At the conclusion of the DCCT, participants returned to the care of their regular health care providers. However, researchers continued to observe the health of more than 90 percent of the DCCT participants in an ongoing follow-up NIDDK effort called the Epidemiology of Diabetes Interventions and Complications Study (EDIC). By continuing to observe these well-characterized patient groups, the investigators hoped to determine whether the interventions that had worked so well to reduce microvascular disease risk might also yield a long-term benefit of reducing CVD.

In the EDIC study, the HbA1c levels of the study groups gradually equalized over time as glucose control in the original conventional therapy group improved, while that of the intensive therapy group worsened. Intriguingly, the EDIC initially found that differences in risk for microvascular complications between the original study groups persisted for at least 8 to 10 years, *even though the difference in HbA1c levels disappeared*. Then, in 2005, EDIC investigators reported for the first time that intensive glucose control during the DCCT trial period could also reduce long-term CVD risks in type 1 diabetes. Twelve years after the DCCT had ended, members of the original intensive therapy control group had a *42 percent lower risk for heart disease and a 57 percent lower risk for non-fatal heart attacks, strokes, or death from a cardiovascular event* compared with those who had been in the standard treatment group.

A similar trial for type 2 diabetes was conducted in the United Kingdom (U.K.) from 1977 to 1997. In the U.K. Prospective Diabetes Study (UKPDS), which was supported in part by NIDDK, more than 4,000 newly-diagnosed type 2 diabetes patients were stratified by body weight and randomly assigned to one of four treatment groups: conventional therapy, primarily through dietary changes, or intensive therapy to lower blood glucose levels to close to normal using one of the following three diabetes medications: (1) insulin; (2) a sulfonylurea drug; or (3) metformin. (Only participants who met the trial definition of overweight could be randomly assigned to primary metformin treatment in the UKPDS.) Like the DCCT, the UKPDS demonstrated that intensive therapy to control blood glucose and lower HbA1c levels could reduce the risk of microvascular disease in people with diabetes. UKPDS results suggested that intensive therapy might also confer a benefit with respect to CVD, but, at the conclusion of the intervention—patients were followed for an average of 10 years—the differences were not statistically significant. Therefore, an important question remained unanswered as to whether intensive control could protect people with type 2 diabetes from CVD.

Long-Sought Information Emerges on Glucose Control and Cardiovascular Disease

Because of its substantial impact on the health and lives of people with diabetes, researchers have long sought effective strategies to prevent or manage diabetic CVD. Several clinical trials had proven that carefully controlling blood pressure and cholesterol levels—both of which contribute to CVD risk—substantially reduces cardiovascular events in people with type 2 diabetes. At the conclusion of the intervention, the UKPDS, the first major clinical trial to examine the effects of intensive glucose control in type 2 diabetes, fell short of proving that improved control of blood glucose levels reduced CVD.

Because the DCCT and the UKPDS trials had proven that good glucose control reduced microvascular complications in both type 1 and type 2 diabetes, subsequent expert guidelines for blood glucose management recommended an HbA1c target of 7 percent, the level of control targeted in UKPDS and

proven to reduce eye, kidney, and nerve complications. Widespread acceptance of those recommendations meant that any subsequent attempt to prove glucose control could lessen CVD must study even more stringent control so that participants would not be put at increased risk of microvascular disease.

During the past decade, several studies were begun to answer this key question, most notably the Action to Control Cardiovascular Risk in Diabetes Study (ACCORD), which is led by the National Heart, Lung, and Blood Institute with NIDDK support. ACCORD was designed to test three treatment approaches to decrease the high rate of CVD among adults with established type 2 diabetes who are at especially high risk for heart attack and stroke. More than 10,000 patients with type 2 diabetes were assigned to one of two regimens for blood glucose control: now-standard therapy designed to attain an HbA1c value of 7.0-7.9 percent, or intensive therapy with the intent of lowering HbA1c levels to below 6.0 percent. After patients had been treated for an average of 3.5 years, the intensive therapy arm was halted 18 months ahead of schedule due to a higher rate of deaths and no significant reduction in cardiovascular events in this treatment group.

Two other studies, an industry sponsored trial (ADVANCE) and the Veterans Administration Diabetes Trial (VADT), also compared the effects of standard and intensive blood glucose control on CVD in participants with longstanding type 2 diabetes similar to the ACCORD participants. Although neither of these studies found increased mortality with intensive therapy, they both failed to find any significant reduction in cardiovascular events.

The results of the three recent trials generated huge interest in the medical community and their full implications are still being explored. Further analyses over the next year may help to clarify some factors, such as patient characteristics and treatment regimens, contributing to the differences, but may not identify the cause of the excess deaths in the ACCORD trial.

While the ACCORD trial demonstrated the danger of intensive glucose management to near normal glucose levels in patients with longstanding type 2 diabetes who were at especially high risk of CVD, it did not address the question of cardiovascular benefit of good glucose control instituted shortly after diagnosis when good control can be achieved with simpler diabetes control regimens. The best evidence of the benefits of early treatment comes from the recently reported long-term follow-up of the UKPDS participants. There were no early adverse effects of intensive glucose control in the newly-diagnosed type 2 diabetes patients studied in the UKPDS. Three-quarters of UKPDS participants were observed for 10 years after the end of the original intervention trial. In 2008, the UKPDS follow-up study reported similar benefits for type 2 diabetes patients as had been seen in EDIC for type 1 diabetes. The intensive therapy groups had persistent reductions in microvascular complications and substantial reductions in risk for heart attack compared to those assigned to standard therapy. Intensive therapy participants also had a lower overall risk of death during the course of the study. In the UKPDS follow-up study, as in EDIC, the HbA1c levels between groups became equal for most of the follow-up period. Thus, a period of intensive diabetes management to control glucose levels appears to confer enduring benefits in terms of reducing diabetic complications—including CVD—even if an individual's glucose control subsequently becomes less stringent. This phenomenon, which has been termed "metabolic memory" or the "legacy effect," provides a powerful motivation for most diabetes patients to maintain their glucose levels as close to normal as possible early in the disease.

One Treatment Approach Is Not Suitable for All People with Diabetes

The results of the DCCT/EDIC and UKPDS represent landmark advances in validating intensive glucose

management as a strategy to prevent microvascular and cardiovascular complications in both type 1 and type 2 diabetes. But ACCORD and other large clinical trials of blood glucose control and cardiovascular risk in type 2 diabetes arrived at a seemingly conflicting conclusion. On the surface, the ACCORD outcome seems at odds with the UKPDS finding that intensive glucose control is protective in terms of reducing cardiovascular risks, including death, in people with type 2 diabetes. However, there are important differences between the studies. UKPDS participants had a median age of 53 years and were newly diagnosed with diabetes at the time of enrollment. In contrast, the ACCORD cohort was older, with an average age of 62 years, and had been living with diabetes for a median duration of 10 years. ACCORD participants were also at especially high risk of CVD, and more than a third had already experienced at least one cardiovascular event before the trial began. Moreover, the ACCORD "intensive" therapy protocol attempted to reduce HbA1c values to "near normal" (i.e., non-diabetic) levels, a considerably more aggressive approach to glucose control than the "intensive" therapy regimens of the UKPDS and DCCT. Viewed together, the results of ACCORD and UKPDS suggest that a *personalized* approach to glucose control in type 2 diabetes might be needed—one that takes into account a person's duration of diabetes, the presence or absence of diabetes complications, risk of low blood glucose, other complicating illnesses and life expectancy, as well as other health, behavioral, and social factors.

Conclusions

The recent results of long-term clinical trials to reduce diabetes complications are expanding our knowledge of the best ways to manage diabetes. Despite some challenges, progress is being made in improving glucose control and reducing both micro- and macrovascular complications related to both type 1 and type 2 diabetes. Further investigation is needed, since no current treatment regimens fully replicate the tightly regulated control of glucose levels found in people without diabetes.

Type 1 and type 2 diabetes are complex chronic diseases that have multiple clinical presentations, variability in their rates of progression, and variability in susceptibility to development of chronic micro- and macrovascular complications. Strategies for controlling blood glucose to prevent complications may need to be modified for different groups of patients or even for a single patient as their disease progresses. Such strategies must also take into account other therapies to manage CVD risks, such as drugs that normalize blood pressure, reduce blood lipid levels, or alter blood coagulation.

As the number of people with diabetes in the U.S. continues to climb, the NIDDK investment in long-term clinical trials to optimize diabetes management will help reduce the burden of CVD and premature death in this large segment of the population. In addition, basic research to understand the phenomenon of metabolic memory will shed light on the way intensive glucose control early in the course of diabetes can pay off in terms of fewer complications years later. In time, it may be possible to reproduce the effects of metabolic memory even in patients with poorly controlled diabetes and, thereby, help all people with diabetes achieve better health and longer lives.

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RNAi-based Therapeutic Strategies for Metabolic and Inflammatory Diseases

Dr. Michael Czech

Dr. Michael Czech is a Professor and Chair of Molecular Medicine and a Professor of Biochemistry and Molecular Pharmacology at the University of Massachusetts Medical School in Worcester, Massachusetts. He received his Ph.D. in Biochemistry from Brown University. Dr. Czech has published nearly 300 papers and has served, or is presently serving, on the editorial boards of dozens of journals. He has also served on the NIH endocrinology study section and on the Howard Hughes Medical Institute review panel. Dr. Czech was the recipient of the Grodsky Basic Research Award from the Juvenile Diabetes Research Foundation International in 1997, the 1998 Elliot P. Joslin Medal in diabetes research, and the 2000 Banting Medal of the American Diabetes Association. The following are highlights from the scientific presentation that Dr. Czech gave to the NIDDK's Advisory Council in May 2008.

Understanding the process by which the body slowly becomes resistant to the hormone insulin, as is the case in type 2 diabetes, is critical to developing effective therapeutics for the disease. Recent research has revealed a link between insulin resistance and the inflammatory response of the immune system. As the body takes in excess calories, fat cells, known as adipocytes, increase in size to store the extra fat. Eventually, the adipocytes become overloaded and begin to release molecules that attract inflammatory cells, specifically macrophages. Macrophages are important in initiating the inflammatory response; they engulf foreign pathogens, such as bacteria or yeast, and secrete molecules that affect the behavior of other immune system cells and that attract additional inflammatory cells to the site of the pathogen. However, inflammation does not only occur when there is an obvious infection. A chronic state of inflammation can occur when macrophages are continually recruited to adipocytes, as in the case of obesity. In this state and with the adipocyte's ability to store fat exceeded, the muscle begins to take up the excess fat. The build up of fat in the muscle disrupts the ability of insulin to stimulate the transport of glucose (sugar) from the blood into muscle, leading to insulin resistance.

Dr. Czech's presentation moved from an initial fundamental discovery to an innovative strategy for its clinical application. He discussed his approach to understanding how cells become resistant to insulin and the role of the inflammatory response in insulin resistance. He shared how his laboratory has utilized a revolutionary technique—ribonucleic acid (RNA) interference, or RNAi—to identify novel molecules critical to these processes. Dr. Czech and his team are exploring the use of this technique as a potential therapy for insulin resistance. Dr. Czech also remarked that this research was made possible by NIDDK's Diabetes Genome Anatomy Project (DGAP), a unique and multi-dimensional initiative for basic research in diabetes. DGAP was designed to facilitate interactions and coordinate a number of investigators at multiple institutions, with projects aimed at understanding the interface between insulin action, insulin resistance, and the genetics of type 2 diabetes.

Using RNAi To Identify Novel Proteins in Insulin Resistance

Dr. Czech and his colleagues sought to identify proteins that mediate the interactions between

adipocytes and macrophages and to understand their role in the balance of blood glucose and fat levels. Such information could reveal new drug targets to break the link between obesity and insulin resistance. To uncover these proteins, the scientists used a technique based on the phenomenon of RNA interference. This technique involves designing small molecules, known as small interfering RNAs or siRNAs, that reduce levels of a specific protein by interacting—or interfering—with the genetic material that encodes the protein, to prevent the protein from being made. The scientists thus could design specific siRNAs to reduce the level of a protein and see whether insulin-mediated glucose uptake was affected. With this technique, they screened hundreds of different proteins in mouse adipocytes to determine whether any had a role in insulin action.

Several specific proteins were identified that Dr. Czech and his colleagues never expected to be involved in insulin resistance. One of these is called MAP4K4 (shorthand for mitogen activated protein 4 kinase). Dr. Czech's laboratory subsequently demonstrated that MAP4K4 blocks insulin-stimulated glucose transport through a mechanism that also involves an inflammatory response protein called TNF-alpha. This positions MAP4K4 at the interface between adipocytes, where MAP4K4 can be found, and macrophages, which secrete TNF-alpha. MAP4K4 is also located in other types of cells, and Dr. Czech's laboratory and others have identified additional roles for this protein in muscle and in macrophages, placing MAP4K4 in three key tissues involved in insulin resistance in obesity.

Developing RNAi as a Potential Therapeutic

Once a role had been identified for MAP4K4 in inflammation and insulin-dependent glucose uptake, Dr. Czech wanted to explore whether targeting this protein, using the power of RNAi, could have therapeutic potential for diabetes. The investigators decided to target levels of MAP4K4 protein in macrophages, rather than in muscle or adipocytes, because they hypothesized that insulin resistance results from the stimulation of inflammation by MAP4K4 in macrophages. In addition, because macrophages—and inflammation—are involved in many diseases, such as rheumatoid arthritis, colitis, inflammatory bowel disease, cardiovascular disease, and atherosclerosis, developing a strategy for therapy in the macrophage might be applied to many other diseases.

Dr. Czech explained that RNA interference as a potential therapeutic may have several advantages over traditional small molecule drugs, which interact with proteins. In traditional drug development there are a relatively limited number of proteins that can be targeted, as the small molecules normally tested as drug candidates are only effective if they can bind (attach) to the targeted protein. By contrast, RNAi works by interfering with genetic material encoding proteins, not the proteins themselves, and scientists think that there may be fewer structural constraints for this type of interaction. With RNAi, therefore, levels of any protein encoded in the human genome could theoretically be targeted and reduced. Second, traditional small molecule drugs can sometimes bind non-targeted proteins. Because siRNAs are extremely specific in their targets, off-target—and potentially toxic—effects can be minimized. Additionally, siRNAs are made from materials that are native to the body and have not shown toxicity thus far in animal models.

As Dr. Czech noted, an ideal therapeutic would be delivered orally for the patient's ease. An orally delivered drug faces many obstacles on its way to the target tissue: it needs to pass through the acidic environment of the stomach, be absorbed by the gut, and enter the bloodstream. An ideal therapeutic would be specifically delivered to the targeted tissues, thereby avoiding any toxicity due to misdelivery. To address these challenges, Dr. Czech took advantage of special cells called "M cells," which are located within the small intestine, and devised a way to get siRNA to these cells.

The M cells constantly sample the digestive cavity of the intestine looking for particles like bacteria and yeast that may have been ingested. Upon finding these, M cells are able to bind the particles, internalize them, and expel them where nearby macrophages are waiting to devour them. With this system, Dr. Czech utilized the normal biology of the intestine to efficiently direct his RNAi therapeutic to the macrophages.

Dr. Czech's laboratory needed to generate a safe vehicle to deliver the siRNA to an animal being studied. Their efforts led to the development of hollow, porous, tiny (micron-sized) shells made of a substance called beta1,3-D-glucan, which is recognized by proteins on both the M cells and the macrophages, permitting these cells to take in the shells. Beta1,3-D-glucan is a non-toxic material made by yeast cells and has been sold as a human dietary supplement for many years. Layering the siRNA within the hollow center of the shell allows five to six layers of siRNA to be put into each of these particles. Therefore, the scientists could use multiple combinations of siRNA at one time and target several different genes, or use one siRNA at a higher dose. Dr. Czech and his colleagues termed these shell particles "GeRPs" or Glucan-encapsulated siRNA particles.

Proof of Principle: Using RNAi To Target MAP4K4 in Animals

This technology required multiple tests to determine whether it could be used as a potential therapeutic in animals. To begin, Dr. Czech and his colleagues needed to ascertain whether the macrophages would even ingest the GeRPs— the first step in this strategy. To do this, the scientists added a fluorescent label to the GeRPs and gave them orally to mice. Using a fluorescence microscope, they were able to see that the macrophages had taken in the GeRPs and that a single macrophage could ingest multiple GeRPs. Another exciting aspect of this technology is that it harnesses the macrophages in the gut to transport the GeRPs. These macrophages are part of the body's lymphatic system, which enables them to travel throughout the body. This prompted Dr. Czech and his colleagues to assess if they could find GeRPs inside macrophages located in various tissues of the mouse body. After 8 days of feeding the mice GeRPs, the scientists observed the fluorescent GeRPs in the lungs, liver, and spleen. From this result, Dr. Czech and his laboratory concluded that they are able to target multiple tissues in the mouse body with this technology.

Dr. Czech's next step was to examine whether GeRPs with siRNA directed to MAP4K4 led to a reduction in the levels of MAP4K4 proteins within the tissues of the mice. In spleen, liver, and lung, the scientists were able to see a reduction in the levels of MAP4K4 as they had hoped. Did this reduction in MAP4K4 protein levels affect the inflammatory response though, as Dr. Czech had predicted? The scientists again fed the mice GeRPs with siRNAs to MAP4K4 and then gave the animals a toxic chemical that mimics a bacterial infection in order to stimulate the inflammatory response. When mice without the siRNAs were given this chemical, their macrophages stimulated an excessive inflammatory response, leading to a very large release of the inflammatory protein TNF-alpha, which was fatal to the animals. However, by feeding the mice siRNA to MAP4K4, Dr. Czech and his colleagues were able to block this storm of TNF-alpha, halting the inflammatory response to the chemical, and protecting the mice. This exciting result demonstrated that the orally administered siRNAs were not only delivered to the correct cell, the macrophage, and carried to multiple tissues, but that these siRNAs also targeted MAP4K4 specifically and altered the mouse inflammatory response.

Does using this technology to target MAP4K4 reduce inflammation in fat tissue and affect insulin-mediated glucose transport into cells? For these preliminary experiments, Dr. Czech and colleagues used obese mice that are highly insulin-resistant and delivered MAP4K4 siRNA-containing GeRPs to the mice by

injection. The scientists looked at various tissues to determine the location of GeRP-filled macrophages and evaluated whether the mice were still resistant to insulin with a test called a "glucose tolerance test." They found, to their surprise, that the fat tissue of these mice was the main tissue that had macrophages with GeRPs in them. This indicated that, in these obese mice, the primary inflamed tissue is the fat tissue— macrophages are largely recruited to this tissue. Dr. Czech and his laboratory also observed a decrease in levels of MAP4K4 protein in the macrophages recovered in this tissue. In addition, these mice were better able to metabolize glucose, indicating that the insulin resistance of these obese mice could be ameliorated. These experiments suggested that delivery of MAP4K4 siRNA to obese mice could have a profound effect on glucose metabolism throughout the body.

Conclusion

Dr. Czech's presentation illustrated the power of RNAi technology to identify novel proteins involved in insulin resistance. These proteins are potential targets for drug therapy, as they are found at the interface between fat cells, muscle, and the inflammatory response. One particular protein, MAP4K4, is especially interesting due to its location in all of these tissues. In addition, Dr. Czech showed his laboratory's approach to using siRNAs as a therapeutic modality. By targeting siRNA to MAP4K4 within the macrophages of a mouse with an innovative delivery vehicle, the scientists were able to both block the inflammatory response and alter the insulin resistance in obese mice. Thus, Dr. Czech and his colleagues have developed a technology to deliver RNAi *in vivo* in mice. They plan to build on the studies to determine whether the therapeutic has a similar result in other animals. Dr. Czech's research reveals the exciting potential for a new method of therapy for numerous diseases, including type 2 diabetes.

PATIENT PROFILE

Charlotte Cunningham

With Type 1 Diabetes, "Time Is of the Essence"

Charlotte Cunningham

Late in the summer of 2005, Lilo Cunningham noticed that her then 10-year-old daughter, Charlotte, was beginning to drink copious amounts of water. This seemed unusual to Lilo because Charlotte was not fond of drinking water. "But no matter where we went, she was always looking for a water fountain," says Lilo. Lilo also noticed that Charlotte was using the bathroom more frequently.

Lilo recognized these changes in Charlotte's behavior as potential symptoms of diabetes. As two of Lilo's sisters have sons with the type 1 form of the disease, Lilo decided not to take a chance. Within days of her observations, Lilo made an appointment with Charlotte's pediatrician and, sure enough, learned that Charlotte's blood sugar level was 680—about seven times above normal.

Charlotte was diagnosed with type 1 diabetes—previously known as juvenile diabetes—a devastating illness that often strikes in infancy, childhood, or young adulthood.

The diagnosis was frightening, but Lilo was able to turn to her sisters for advice. In addition to offering many practical suggestions for dealing with diabetes on a day-to-day basis, one of Lilo's sisters, who is very active in the Juvenile Diabetes Research Foundation International (JDRF), informed her that several diabetes research trials were under way. She suggested that the Cunninghams might want to investigate these trials for Charlotte.

Because the Cunninghams were informed of several clinical trials shortly after Charlotte's diagnosis, she was eligible to participate in a clinical trial specifically designed for newly-diagnosed patients. The therapy being tested in this trial may slow down the progression of the disease, which could reap long-term benefits for patients and make it easier for them to control their blood sugar levels.

"The more we can slow the progression of this disease and keep Charlotte healthy, the better chance she has of leading a longer, healthier life."

Controlling blood sugar levels is critical. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT) demonstrated that intensive blood sugar control offers remarkable long-term benefits when it comes to preventing or delaying complications frequently associated with type 1 diabetes, including eye, nerve, kidney, and cardiovascular disease.

Charlotte, now 13 years old and 3½ years post-diagnosis, shows no signs of complications from diabetes. "Time is of the essence," says Lilo. "The more we can slow the progression of this disease and keep Charlotte healthy, the better chance she has of leading a longer, healthier life."

PATIENT PROFILE

About the Study

Type 1 diabetes occurs when a person's immune system mounts a misguided attack and destroys the insulin-producing beta cells found in the pancreas. Insulin is critical for the body to absorb sugar from the blood and to use it for energy. Those with type 1 diabetes need daily administration of externally-supplied insulin, either by injection or with a pump, and must monitor their blood sugar levels vigilantly. Researchers have discovered, however, that many individuals diagnosed with type 1 diabetes still make detectable amounts of insulin, even many years after they are diagnosed. The DCCT also showed that people with type 1 diabetes who still made some of their own insulin had fewer long-term disease complications, as well as reduced incidents of dangerously low blood sugar (hypoglycemia) from administration of too much insulin. These observations suggest that preserving patients' remaining beta cell function, so that they still produce some of their own insulin, could have dramatic, long-term health benefits.

"We had an incredibly positive experience with Charlotte's study. We were exposed to so many people who know so much about this disease—we learned so much!"

The trial in which Charlotte is participating is trying to do just that. A previous NIDDK-supported clinical trial indicated that an antibody, called hOKT3gamma1(Ala-ala) or "anti-CD3," halted the destruction of insulin-producing beta cells in a small number of newly-diagnosed patients. Anti-CD3 alters the signal that triggers the disease-causing immune cells to attack the insulin-secreting cells. Charlotte is participating in a trial where researchers are determining if an additional treatment of anti-CD3 will provide further benefit, beyond that of the single treatment. This trial is being conducted by the Immune Tolerance Network, which is led by the National Institute of Allergy and Infectious Diseases, in collaboration with the NIDDK's Type 1 Diabetes TrialNet. Both networks also receive funding from the Special Statutory Funding Program for Type 1 Diabetes Research. Because one of the requirements for participation in this particular trial was that patients enroll within 8 weeks of their diagnosis, the Cunninghams are very grateful that a family member counseled them to act quickly after Charlotte's diagnosis.

"We were fortunate that Charlotte was diagnosed so early and was able to participate in this trial," says Lilo. "As a result, she's perhaps making more insulin than the average person in the early stages of diabetes and is doing very well."

The trial requires Charlotte to be infused daily over a 14-day period with the anti-CD3 antibody. Each daily infusion takes between 15 to 30 minutes, and is administered into Charlotte's upper arm. Charlotte received this 14-day set of infusions two times; the second treatment followed 19 months after the first. Charlotte returned to the trial site every 3 months in between the treatments and for 12 months following the second treatment. These visits were to monitor her response to the treatment and included a physical examination, a blood test, and a test to measure her insulin response. Except for a rash between her fingers, which lasted only 1 day, Charlotte has experienced no side effects from the treatment.

When asked about her overall experience in the trial study, Charlotte responded, "It was very cool." Not the typical response one would expect from an adolescent, but Charlotte has handled her diabetes extremely well from the beginning.

Lilo and Charlotte's Message: Don't hesitate. Act quickly.

When it comes to diabetes, Lilo and Charlotte's message to others is clear and simple: At the first sign of symptoms, do not hesitate; act quickly.

PATIENT PROFILE

"If you have any suspicions or notice anything wrong with your child, go for a blood test [at your pediatrician's office] and follow up immediately," says Lilo. "If this study succeeds in allowing Charlotte to retain the ability to produce some of her own insulin, even for a little while longer than she might have otherwise, it will help to delay, reduce, and possibly even prevent the secondary complications that often accompany type 1 diabetes." "And make sure you check your blood sugar level regularly," adds Charlotte.

"Having diabetes hasn't really affected me much when I'm doing sports…my coaches are very understanding and let me do what I need to do to take care of myself."

Lilo has not observed symptoms in other family members, but that does not mean she was going to take chances. The Cunninghams enrolled their two other children, Charlotte's 16-year-old brother and 19-year-old sister, in a study as well—the TrialNet Natural History Study. This study is screening relatives of people with type 1 diabetes to determine what level of risk these family members have for developing the disease. These studies are being conducted to learn more about the causes and indicators of risk for the development of type 1 diabetes. So far neither one of Charlotte's siblings appears to be at increased risk. "But if either of them should show signs of the disease, I would enroll them in a clinical trial in a heartbeat," Lilo says. "We had an incredibly positive experience with Charlotte's study. We were exposed to so many people who know so much about this disease—we learned so much!" When asked her thoughts on participating in the trial, Charlotte proudly says, "I'm an example of how diabetes research is helping people."

About Charlotte

Since February 2008, Charlotte's need for injected insulin has increased dramatically. According to Lilo, it is hard to say exactly what is going on. "Charlotte is in the midst of puberty, which could mean her body is requiring more insulin because of hormonal changes," she says. Nineteen months after her first treatment, Charlotte received her second and final 14-day infusion as part of the trial. The good news is that, even though Charlotte needs more external insulin, tests performed in May 2008 (12 months after Charlotte's last treatment and nearly 3 years after her initial diagnosis) indicate that she is still producing some insulin. Because her need for external insulin is increasing, Charlotte is exploring the possibility of using an insulin pump, a portable device that injects insulin at programmed intervals. She says she is excited about the prospect of using the pump.

If anything, Charlotte's life has become more active, rather than less, since being diagnosed with diabetes. Prior to her diagnosis, Charlotte played tennis and basketball. Now she has added surf boarding, lacrosse, and softball to her repertoire of physical activities. "Having diabetes hasn't really affected me much when I'm doing sports," she says. "I need to make sure my blood sugar count is okay both before and while I'm playing, but my coaches are very understanding and let me do what I need to do to take care of myself."

In the meantime, at the time this story was written, Charlotte was preparing to go to summer camp with 70 of her peers, all of whom have diabetes. She has been to the camp twice before and says she likes it a lot. "We meet with meal planners and check our blood sugar regularly, but mostly it's a regular, fun camp," Charlotte explained. Like any 13-year-old, Charlotte simply wants to lead as active and normal a life as possible.

PATIENT PROFILE

Verner Thomas

DEPLOY Lifestyle Interventions Delay, Prevent Type 2 Diabetes

Verner Thomas

At age 61, retired and overweight, Verner Thomas joined the YMCA to try to improve her health. On one of her visits, she was handed a brochure asking if she'd like to participate in something called the "Diabetes Education & Prevention with a Lifestyle Intervention Offered at the YMCA," or DEPLOY. DEPLOY is testing a diabetes prevention model in which YMCA employees are trained to help people who have risk factors for type 2 diabetes lose weight and increase their physical activity.

With support from NIDDK and in cooperation with the YMCA, the DEPLOY program is building on previous NIDDK-supported research that demonstrated that, in people at high risk, an intensive lifestyle intervention can be an extremely effective means of preventing or delaying the onset of type 2 diabetes.

"My daughter is really proud of me," says Verner. "She says 'Momma, you look better and feel better. You're just a much more vibrant person. I want you to keep this up!'"

And Verner is a reaffirmation of those findings.

Prior to involving herself in the program, Verner's blood sugar and weight strongly indicated that she was at high risk of developing type 2 diabetes within the next 1 to 2 years. Additionally, her cholesterol levels were abnormally high. Today, 2 years after being introduced to the DEPLOY program, Verner exercises regularly, eats a more disciplined diet, and in general is more conscious of the lifestyle that she leads. As a result, she has lost weight, lowered her blood sugar levels, improved her cholesterol levels, and, best of all, has remained diabetes free.

"My daughter is really proud of me," says Verner. "She says 'Momma, you look better and feel better. You're just a much more vibrant person. I want you to keep this up!'"

After speaking with Verner, one gets the feeling that her daughter was preaching to the choir. Ms. Thomas is well aware of the positive changes she's made in her life as a result of DEPLOY.

Type 2 Diabetes—Reducing the Burden

Diabetes is a chronic, common, and costly disease that is robbing many Americans of good health and quality of life. Type 2 diabetes—once known as adult-onset diabetes, or non-insulin-dependent diabetes

PATIENT PROFILE

mellitus—is the most common form of the disease. It primarily affects adults, but it can develop in childhood and adolescence. Older age, overweight, and inactivity are strong risk factors for type 2 diabetes; heredity plays an important role as well. People with diabetes have blood sugar levels that are above normal. Over the years, high blood sugar damages nerves and blood vessels, leading to serious health complications such as heart disease, stroke, blindness, kidney disease and kidney failure, the need for lower limb amputations, and gum infections.

Ominously, Verner is hardly alone when it comes to being at high risk for type 2 diabetes. People who, like Verner did, have blood sugar levels higher than normal, but not high enough to be classified as diabetes, are considered to have "pre-diabetes." In addition to 23.6 million Americans who already have type 2 diabetes, the Centers for Disease Control and Prevention estimates that at least another 57 million have pre-diabetes, and thus are at high risk of progressing to type 2 diabetes.

Importantly, there is a window of opportunity to reverse course on the way to developing type 2 diabetes. Spearheaded by the NIDDK, the landmark Diabetes Prevention Program, or DPP, was a clinical trial that showed that in overweight people with pre-diabetes, type 2 diabetes can be prevented or delayed through use of the diabetes medication metformin, or through a lifestyle intervention leading to moderate weight loss through diet and exercise. The immense success of the intensive lifestyle intervention, which showed a 58 percent reduction in risk of developing diabetes, has led to new research studies and programs testing ways to effectively translate these results into interventions that can be widely and effectively implemented to prevent diabetes in those at risk.

Translating DPP with the DEPLOY Program

Verner's mother had diabetes, as did her grandmother. She has a family history on her father's side of high blood pressure, as well as a history of obesity on both sides of her family. Verner also understands that, as an African American, she has a 1.8-fold increased risk of developing type 2 diabetes compared to non-Hispanic whites.

"This program (DEPLOY) makes you aware of what you should be doing and what you're not doing to protect yourself against type 2 diabetes," says Verner. "It motivated me to take better care of myself—how I eat and exercise….I wasn't as faithful in my exercising until I got into the program."

Operating out of local YMCAs, and led by primary investigator Dr. Ronald Ackermann of the Indiana University School of Medicine, DEPLOY takes groups of 8 to 12 people who, like Verner, have pre-diabetes and other risk factors, and puts them through a series of classroom-style meetings that focus on knowledge building and skill development to help them set goals, self-monitor, and problem-solve around their pre-diabetes. Major goals of the program include a 5 to 7 percent reduction in baseline weight, and 150 minutes per week of moderate level physical activity similar to brisk walking. The program is based on the lifestyle intervention that proved so effective at delaying or preventing type 2 diabetes among participants in the DPP.

Verner first started with DEPLOY in January 2006. Since then, in addition to remaining diabetes free, her weight has gone from 219 pounds down to 177, and her blood pressure has gone from 133/90 to 110/72 (normal is 120/80 or lower). Her body mass index (BMI) has also dropped from 33 to 29. BMI is a measure of weight relative to height. A BMI of 30 or more in an adult is considered obese. Verner thus is no longer obese—a significant achievement. She remains determined to get her BMI still lower. But in all categories, including her sugar and cholesterol levels, Verner's numbers are heading in the right direction. Her fasting blood sugar has fallen from 111 to 90 milligrams per deciliter, meaning she no longer has pre-diabetes—and for that she is grateful.

PATIENT PROFILE

"I feel healthier and stronger today than I did 2½ years ago," says Verner. "I've gotten a lot of compliments since I lost the weight, and psychologically I feel better. I'm more open, social, and outgoing," she adds with a satisfying chuckle.

She admits that she also still faces some challenges. "My problem is sweets," says Verner. "When I'm under stress I fall back on them." But she says that for the most part she's been able to keep her sweet tooth under control, as well as the rest of her diet.

To meet their goal of moderate weight loss, participants in DEPLOY are counseled to increase physical activity and to reduce their intake of fat and total calories. The diet and exercise interventions are flexible and sensitive to individual, cultural, and community differences where they are implemented. As part of her diet, Verner eats fish occasionally, and in addition, she says, "I only eat one egg a month. I get my protein from peanut butter, beans, and meat substitutes."

Looking to the Future

Researchers are making major discoveries in how to predict who will develop type 2 diabetes and its complications; how to personalize individual treatments; and how to use this information to preempt disease onset and development of complications (see box). Public health efforts founded on these discoveries can hopefully help to reduce the burden of type 2 diabetes and its complications in the future. Research programs such as DEPLOY—and the hard work and dedication of the participants—are an important part of making this future real for people at risk for type 2 diabetes.

DEPLOY has a strong fan in Verner: "I'm dedicated to maintaining my regime of eating healthily and exercising regularly," she says, and she is sure that DEPLOY helped point her in that direction.

PATIENT PROFILE

The Beneficiary of Decades of Research
Verner Thomas and the millions of other Americans who are either at high risk or already have type 2 diabetes are the beneficiaries of decades of research.

Thirty years ago there were no proven strategies to prevent type 2 diabetes or its complications, and the only treatments, now obsolete, caused dangerously low blood sugar reactions and weight gain in patients.

Today, as a result of research, diabetes is better understood, new and more effective treatments are available, and type 2 diabetes and its complications can be delayed and in some cases, even prevented. For example:

- Researchers now know that obesity is a strong risk factor for type 2 diabetes, and have a new understanding of the molecular links between obesity and insulin resistance, a condition that prevents the body from effectively using insulin.
- Risk factors other than obesity have been identified and can be targeted.
- Newly-identified diabetes genes will enhance researchers' ability to identify and intervene in those at risk.
- Based on clinical research demonstrating the health benefits of early detection and therapy, Medicare now covers testing for diabetes.
- New drug development has been aided by an NIH-supported clinical trial that validated an indicator, called hemoglobin A1c, that reflects average blood sugar control over a 2 to 3 month period.
- New oral medications that target the specific metabolic abnormalities of type 2 diabetes are available.
- The Diabetes Prevention Program (DPP) demonstrated that type 2 diabetes can be prevented or delayed in those at risk. The benefits of the DPP were seen in all ages, all racial and ethnic groups, in women with history of gestational diabetes, and in people with diabetes risk genes.
- The landmark DPP findings are being actively disseminated to the public by the National Diabetes Education Program.
- Kidney disease resulting from diabetes can now be detected earlier by standardized blood tests to estimate kidney function and monitor urine protein excretion.
- With timely laser surgery and appropriate follow-up care, people with advanced eye disease related to diabetes can reduce their risk of blindness by 90 percent.