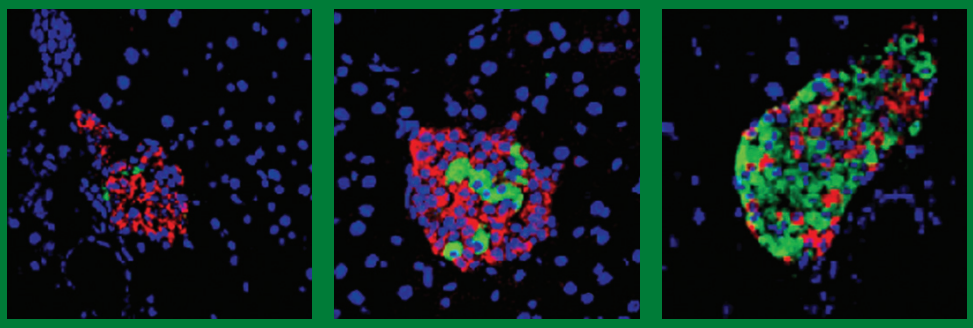

Research highlighted in this chapter demonstrates that pancreatic glucagon-producing alpha cells could convert to become insulin-producing beta cells. These images show alpha cells (red), beta cells (green), and other pancreatic cell types (blue). The image on the left shows a pancreas from a diabetic mouse containing only a few green beta cells. Over time, the mouse regenerated its beta cells, as shown by more green beta cells after 1 month (middle panel) and 10 months (right panel). Importantly, the researchers found that the new beta cells arose from alpha cell conversion. Identifying ways to replace insulin-producing beta cells is important for both type 1 and type 2 diabetes, and this new insight about the ability of one pancreatic cell type to convert to another can inform future research toward developing cell-based therapies for people with diabetes.

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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, these diseases and conditions affect many millions of Americans and can profoundly decrease 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 25.8 million people in the U.S.—or 8.3 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, in working-age adults, 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 and therefore not receiving therapy.[1]

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that 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 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 insulin-producing beta cells of the pancreas. If left untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. 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 to near the normal levels achieved by functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working to develop beta cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 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 the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.[3] Type 2 diabetes occurs at elevated rates among minority groups, including African Americans, Hispanic Americans, American Indians, and Native Hawaiians.[1]

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

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[1] *2011 National Diabetes Fact Sheet. Centers for Disease Control and Prevention. Atlanta, GA.*

[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.*

As a result, blood glucose levels rise, and at first the pancreas produces more insulin to compensate. Gradually, however, the pancreatic beta cells lose their capacity to secrete insulin, and the timing of insulin secretion becomes abnormal. Treatment approaches for controlling glucose levels include diet, exercise, and oral and injected medications, with insulin often required as the disease progresses. There are also an estimated 79 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.[1] 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 type 2 diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight. Moreover, follow-up research has shown that this benefit of reduced diabetes risk can persist for at least 10 years.

Type 2 diabetes was previously called "adult-onset" diabetes because it is 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 than those who develop the disease later in life. 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, patients may find it increasingly difficult to strictly control their blood glucose levels and thus prevent or delay the development of complications. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the U.S.

The NIDDK is supporting research to better understand metabolism and 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

Mapping Gene Regulatory Sites To Understand Islet Biology and Diabetes: Scientists have developed a new technique for identifying regions of the genome that influence gene activity and generated insight into how a specific sequence variant can affect risk for type 2 diabetes. Regions of the genome called "open chromatin" contain genes and regulatory DNA elements that are actively being used within a particular cell type. Using a new technique, the investigators isolated open chromatin from human pancreatic islet cells, which produce insulin and other important hormones, and created a map of these sites in the genome. They hypothesized that islet-specific open chromatin was likely to contain sequences that influence the activity of islet-specific genes and, therefore, sequences that may be associated with diabetes risk. They found that most islet-specific open chromatin sites were in or near genes with known functions in islets. In addition, they compared previously identified type 2 diabetes-associated DNA sequence variants to their map and discovered that a number of disease-associated variants are linked to islet-specific open chromatin sites.

Notably, a variant in the gene *TCF7L2*, which has been consistently associated with type 2 diabetes across diverse ethnic groups, was determined to overlap with an islet-specific open chromatin site. This suggests that the disease-associated variation may regulate the activity of the gene since it is not within the part of the gene that codes for a protein. The scientists then demonstrated that the risk-associated sequence variant was more likely than the non-risk sequence variant to be found in open chromatin, meaning that people with the risk version may produce more of the protein encoded by *TCF7L2*. Indeed, they found that the risk-associated variant can affect gene activity. These results suggest that the risk variant may affect activity of *TCF7L2* by

opening the site to allow more of the protein to be made and provide a potential mechanism for type 2 diabetes susceptibility.

The islet-specific map generated in this study provides a new tool for understanding the regulation of genes important for islet cell biology and for narrowing genomic locations likely to harbor unidentified sequence variants that influence type 2 diabetes susceptibility. This study also validates a new technique for identifying regions of the genome that regulate gene activity. The technique provides an additional means to move beyond identification of disease-associated sequence variants to an understanding of their influence on disease risk, particularly for variants that do not affect the code for a protein. Determining the mechanism by which genetic factors contribute to diabetes is key to understanding both type 1 and type 2 diabetes, identifying individuals at risk, developing and testing prevention strategies, and generating more personalized interventions for people with or at risk for disease.

Gaulton KJ, Nammo T, Pasquali L, et al. A map of open chromatin in human pancreatic islets. Nat Genet 42: 255-259, 2010.

New Discoveries on the Genetics of Blood Glucose Regulation and Insulin Resistance: New genomic technologies have provided a wealth of data on the complex genetic underpinnings of diseases like type 2 diabetes. For example, researchers have used a technology called the genome-wide association (GWA) study to compare single nucleotide polymorphisms (SNPs) throughout the genomes of thousands of people with and without the disease to identify common variants that affect the likelihood of developing diabetes. Recently, researchers took a slightly different approach to shed still more light on diabetes genetics. In people without diabetes, pancreatic function tightly controls the level of glucose present in the blood, ensuring that there is always enough glucose that cells will have an adequate supply, but not so much that the excess is toxic. The new research, however, proceeds from the observation that, even among people who do not have diabetes, there is variation in blood glucose and insulin levels.

For this study, a consortium of investigators re-examined data from dozens of prior GWA analyses, focusing on the "control" populations—those without diabetes. By grouping the participants according to several different measures of metabolic function, they identified a total of 16 genetic locations that appear to have an effect on fasting blood glucose levels, and two that influence fasting insulin levels and insulin resistance, all in people without diabetes. Four of these genetic locations previously had been associated with type 2 diabetes. This study also analyzed potential associations of the variants with type 2 diabetes, and found that five of the 16 gene regions also are linked to risk for the disease. This collaborative study helps define a new approach to identify diabetes risk genes. Further analysis of the genes near the variations found in these studies will help better explain how blood glucose levels are controlled in health and disease. By better understanding the molecular control of healthy blood glucose levels, scientists may one day be more able to predict type 2 diabetes with precision, tailor treatment to people in particular risk categories, and develop improved therapies to help people with diabetes keep their blood glucose at an appropriate level.

Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42: 105-116, 2010.

Identification of Novel Genetic Risk Factor for Type 1 Diabetes Using Analysis of Gene Networks: Scientists have identified a novel genetic variant associated with type 1 diabetes risk that regulates a network of immune system genes. GWA studies have identified many common genetic variants that are associated with human disease. For many of these variants, however, it is not known how the specific variation affects disease risk. This may be true particularly if the variant is not located in a gene or the variant does not appear to affect the activity of the gene in which it is located. To translate these associations with disease to discovery of their roles in disease risk, scientists are investigating whether "gene networks"—sets of genes that are co-regulated—are affected by these genetic variants. In the cell, genes that have related functions tend to be co-regulated; that is, they are turned on or off in similar ways with comparable timing.

In this study, scientists analyzed data from rat tissues to identify gene networks and correlate changes in the regulation of these networks with genetic variants.

They identified a novel gene network that includes a number of genes involved in the immune response to viruses. This network is regulated by the protein factor Irf7 and designated the Irf7-driven inflammatory gene network (IDIN). The scientists demonstrated that the rat IDIN was influenced by a genetic variation on chromosome 15 in a gene known as *Ebi2*, which encodes a protein involved in migration of a specific type of immune cell.

To translate this finding to humans, the scientists looked to see whether the IDIN similarly exists in human immune cells. Not only did they discover the human *IRF7*-driven network, but they also found that genetic variants affecting the human form of *EBI2* had an effect on the IDIN gene network. Since the human IDIN contained a well-characterized type 1 diabetes susceptibility gene, the researchers hypothesized that other genes in the IDIN might be associated with type 1 diabetes risk. They found that genetic variants near IDIN genes were more likely to be associated with type 1 diabetes than were genes not in the network, and they identified a new genetic variant for the disease near *EBI2*. Therefore, this exciting study implicated IDIN genes and their regulation in type 1 diabetes and elucidated the role of *EBI2* in disease risk. Uncovering the functional effects of genetic variation associated with type 1 diabetes will not only increase understanding of how this disease develops, but will illuminate key targets for the development of therapies to prevent the disease.

Heinig M, Petretto E, Wallace C, et al. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature 467: 460-464, 2010.

BETA CELLS AND DIABETES

Protein Found That Drives Development of Insulin-Producing Cells: Finding ways to reduce or eliminate the burden of injected insulin therapy for people with type 1 diabetes and some with type 2 diabetes is an important goal of diabetes research. One approach to eliminating the dependency on injected insulin is to replenish a person's insulin-producing beta cells. Stem cells or other types of cells that could be reprogrammed to produce insulin may represent a good source of replacement tissue, but to tap their potential it is critical to understand the developmental program that creates a functional beta cell. New research uncovered a key factor necessary for making insulin-producing beta cells in both humans and mice. Previous research had identified a protein that helps trigger embryonic development of pancreatic islets, which contain beta cells and other cell types. Scientists now have found another key protein needed for the subsequent development of distinct islet cell subtypes. Mice lacking the newly identified protein—called Rfx6—can make islets, but these islets do not contain insulin-producing cells. They also fail to make some other hormones normally made by the pancreas. Interestingly, the scientists also found that a rare form of neonatal diabetes is associated with mutations in the human gene that produces the Rfx6 protein, suggesting that Rfx6 plays a critical role in beta cell development in humans as well as mice. Researchers now know they will have to ensure that Rfx6 is present in order to successfully generate beta cells from some other cell type for transplantation into people with diabetes.

Smith SB, Qu H-Q, Taleb N, et al. Rfx6 directs islet formation and insulin production in mice and humans. Nature 463: 775-780, 2010.

Alpha Cells Take on New Identity To Overcome Diabetes: Researchers discovered that pancreatic glucagon-producing alpha cells could convert to insulin-producing beta cells in a mouse model of diabetes. Beta cells are destroyed by the immune system in people with type 1 diabetes and may not function normally in people with type 2 diabetes. Identifying ways to replace beta cells to restore the body's insulin-producing capacity would benefit people with type 1 or type 2 diabetes, and is a major goal of research.

Toward this goal, scientists examined beta cell regeneration in a mouse model of diabetes. Using a combination of genetic engineering and chemical treatment, the researchers destroyed nearly all of the beta cells in the mice, resulting in the development of diabetes in the animals and dependency on injected insulin for their survival. The scientists found that, over time, the beta cells regenerated; after 5 months, the animals produced enough of their own insulin to survive without external insulin treatment. Further experiments showed that the source of the new beta cells was the

alpha cells, rather than, for example, the few remaining beta cells. This insight suggests that it may be possible to develop therapies to promote conversion of alpha cells to beta cells to restore insulin production in people with diabetes. Further research is needed to determine if this conversion of one pancreatic cell type to another can occur in humans, and how to protect new beta cells from immune system attack in type 1 diabetes. But, the finding opens up intriguing new avenues for research toward cell replacement therapy for diabetes.

Thorel F, Népote V, Avril I, et al. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 464: 1149-1154, 2010.

Regulating a Regulator of Pancreas Development: New research has shed light on the regulation of a key protein involved in pancreas development and function. The protein, Pdx1, is required for pancreas development in mice and humans; mutations in the *Pdx1* gene, which can decrease the levels of Pdx1 protein, are associated with diabetes in humans. Because proper amounts of Pdx1 protein are important for its role in the pancreas, scientists examined how levels of the protein are controlled. By studying cells grown in the laboratory, they discovered that another protein, Pcif1, targets Pdx1 for degradation. To understand what effect this has in the whole animal, they examined mice that were genetically engineered to have low levels of Pdx1. These mice had several conditions associated with diabetes or risk for the disease, including impaired glucose tolerance and insulin secretion, as well as reduced mass of their insulin-producing beta cells. Using genetic techniques, the scientists demonstrated that if they also reduced levels of Pcif1 in these mice, levels of Pdx1 increased to normal—because not as much Pcif1 was present to target Pdx1 for degradation. This normalization was associated with improved beta cell survival and increased beta cell mass, which in turn led to improved glucose tolerance and insulin secretion. In other words, signs of diabetes observed in mice with reduced Pdx1 levels were relieved by concurrent reduction of Pcif1. The research provides new insights into how Pdx1 protein levels are controlled and suggests that Pcif1 regulation of Pdx1 could be a therapeutic target for treating diabetes.

Claiborn KC, Sachdeva MM, Cannon CE, Groff DN, Singer JD, and Stoffers DA. Pcif1 modulates Pdx1 protein stability and pancreatic beta cell function and survival in mice. J Clin Invest 120: 3713-3721, 2010.

ADVANCING TECHNOLOGY TO MANAGE DIABETES

Continued Progress in Glucose Monitoring Technology—Implantable Glucose Sensor: New research is paving the way toward less burdensome glucose monitoring for people with diabetes. People with type 1 diabetes must monitor their blood glucose levels and administer insulin to keep glucose levels in a healthy range. Day-to-day monitoring is most commonly achieved through multiple daily finger sticks alone; some people now also use continuous glucose monitors in combination with finger sticks. While these methods are valuable tools for helping people with diabetes stay healthy, they entail a fair amount of patient effort and discomfort. Therefore, researchers are working to advance technologies that can minimize patient burden while sustaining the health benefits of frequent glucose monitoring.

In a recent report, a team of bioengineers described testing of an implantable glucose sensor that monitors tissue glucose and reports data to an external wireless receiver. When implanted into pigs, the system functioned continuously for over a year. The implanted sensor also worked when tested for several months in pigs that were made diabetic through administration of a chemical that is toxic to the insulin-producing pancreatic beta cells. These experiments showed that the sensor remained accurate whether glucose levels were high, low, or normal, and helped gauge the speed with which it detects rising or falling blood glucose. The scientists plan to conduct clinical trials to test the sensor in people. The system does not automatically deliver insulin, so patients would still need to administer insulin based on the sensor readings. However, these results are encouraging because an implantable device not only could reduce the need for finger sticks, but also potentially be used in the future as part of an "artificial pancreas" to automate glucose sensing and insulin delivery.

Gough DA, Kumosa LS, Routh TL, Lin JT, and Lucisano JY. Function of an implanted tissue glucose sensor for more than 1 year in animals. Sci Transl Med 2: 42ra53, 2010.

AUTOIMMUNITY IN TYPE 1 DIABETES

It's All in the Presentation—Type 1 Diabetes and the Display of Insulin to Immune Cells: Scientists discovered that a variant of an immune system molecule may contribute to type 1 diabetes by enabling an aberrant immune reaction against insulin. Genetic variation in the *HLA* genes, which encode a key immune recognition protein, accounts for a large proportion of the genetic risk for type 1 diabetes in humans. However, little is known about how this variation leads to autoimmunity, in which T cells of the immune system destroy the insulin-producing beta cells in the pancreas, resulting in type 1 diabetes. Previous research identified a specific fragment of the insulin molecule as important to the development of type 1 diabetes in a mouse model of the disease. The immune recognition protein binds this fragment and "presents" it to T cells. If T cells recognize insulin presented in this way in an organ called the thymus, then those cells are destroyed because the body normally tries to prevent immune reactions against itself. If T cells that recognize "self" are not destroyed in this process, then they are released throughout the body and could attack the insulin-producing beta cells.

By studying how the specific insulin fragment binds to different sites on the variant immune recognition protein in mice, the scientists noted a surprising finding. Rather than observing strong binding between this variant immune protein and the insulin fragment, as had previously been suggested, they observed that weaker binding led to activation of T cells involved in autoimmunity. The scientists speculated that this weaker binding did not permit adequate presentation of the insulin fragment to the T cells in the thymus and therefore allowed the T cells to escape the normal mechanism that should have destroyed them. Further research is necessary to determine whether a similar mechanism is associated with type 1 diabetes in humans. If it is, then this finding could present an exciting opportunity to intervene in the immune process to prevent the disease or slow its progression.

Stadinski BD, Zhang L, Crawford F, Marrack P, Eisenbarth GS, and Kappler JW. Diabetogenic T cells recognize insulin bound to IA^{g7} in an unexpected, weakly binding register. Proc Natl Acad Sci USA 107: 10978-10983, 2010.

Novel Immune Target for Preventing or Treating Type 1 Diabetes: Researchers in a clinical trials network—Type 1 Diabetes TrialNet—reported that a drug that destroys immune system cells called B lymphocytes preserved the function of insulin-producing beta cells in people newly diagnosed with type 1 diabetes for 1 year. Scientists have known that type 1 diabetes is caused by aberrant immune system destruction of insulin-producing cells, and have implicated immune cells called T lymphocytes (or T cells) in this attack. It had not been clear, however, whether B lymphocytes (or B cells) were involved or could be targets for therapeutic approaches. Scientists tested whether destroying B lymphocytes with four separate infusions of the drug rituximab shortly after type 1 diabetes diagnosis could slow progression of the disease. After 1 year, people who received the drug produced more insulin, had better control of their diabetes, and did not have to take as much insulin to control their blood glucose levels compared to people receiving placebo. However, at 2 years, the effect of the treatment dissipated. Because of the side effects associated with this immunosuppressive drug, the risk to benefit ratio would not suggest that rituximab be used as a therapy for people with type 1 diabetes. Nonetheless, the finding is very important because it demonstrates that B lymphocytes may be a key target for type 1 diabetes prevention or treatment. Rituximab treatment results in a general depletion of B lymphocytes, and the effect of the drug was lost when the depletion ended; it would be unhealthy for B lymphocytes to be chronically depleted (through additional infusions of the drug) because these cells are a part of a functioning immune system. Thus, this study suggests that it may be possible to prevent or treat type 1 diabetes more safely by identifying ways to target the specific B lymphocytes involved in the disease without depleting B lymphocytes more generally.

Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med 361: 2143-2152, 2009.

COMBATING TYPE 2 DIABETES RISK FACTORS IN YOUTH

Results of a Middle School Intervention To Lower Risk of Diabetes: New research shows that

an intervention program in middle schools lowered the obesity rate in a group of students at particularly high risk for type 2 diabetes, but did not have a greater impact on the overall rate of obesity and overweight than was observed in control schools. Type 2 diabetes is an emerging health problem in youth, particularly minority youth, being driven by the obesity epidemic.

To address the problem, the NIDDK-led HEALTHY clinical trial examined whether a middle school-based intervention could lower risk factors for type 2 diabetes. The study was conducted in schools with a high enrollment of minority children and youth from low-income families. Conducted for 3 years—from the beginning of the sixth grade to the end of the eighth—the HEALTHY study involved 4,603 students attending 42 U.S. middle schools in seven areas of the country. Each school was randomly assigned to implement the intervention program or to serve as a comparison (control) school. The intervention program involved changes in school food services; longer, more intense periods of physical education; and classroom activities to promote healthful behavior changes.

The intervention was found to lower the obesity rate in students who started out overweight or obese in sixth grade, a group of children who would otherwise be at higher risk of future type 2 diabetes than the others in the study. Surprisingly, however, schools that implemented the program did not differ from comparison schools in the study's primary outcome—the combined prevalence of overweight and obesity—which had declined by 4 percent in both the intervention and control schools by the end of the 3-year study. One possible explanation is that comparison schools may have independently implemented healthful changes to the school environment because of increased awareness about the problem of childhood obesity fostered by the study. Future research will examine what changes in policy may have been implemented in the comparison schools. The HEALTHY results are important for informing future school-based efforts to reduce overweight and obesity in children.

The HEALTHY Study Group. A school-based intervention for diabetes risk reduction. N Engl J Med 363: 443-453, 2010.

TESTING TREATMENT APPROACHES FOR TYPE 2 DIABETES

Inexpensive, Generic Drug Improves Blood Glucose Control in People with Type 2 Diabetes: Researchers have discovered that the drug salsalate helped people with type 2 diabetes control their blood glucose levels. Salsalate is an inexpensive, generic anti-inflammatory drug that is chemically similar to aspirin, but causes fewer stomach problems. It has been used safely for decades to treat people with arthritis. Because research is showing that metabolic conditions, including type 2 diabetes, are associated with chronic inflammation, scientists tested whether this anti-inflammatory drug could effectively treat people with type 2 diabetes. In the first phase of the Targeting INflammation with SALsalate in Type 2 Diabetes (TINSAL-T2D) clinical trial, 108 people were randomly assigned to four different treatment regimens: one group received placebo, and three groups received different doses of the drug. All participants continued their regular diabetes treatment regimen during the trial. After 3 months, people taking salsalate had lower blood glucose and triglyceride levels on average compared to people taking placebo. Some participants experienced adverse changes such as increased excretion of protein in the urine and higher levels of LDL (bad) cholesterol. Thus, researchers are conducting a longer, larger trial to further test salsalate—knowledge that is needed to further evaluate the relative benefits and risks of the drug. With more research, salsalate may prove to be an inexpensive way to help treat the millions of people with type 2 diabetes in the U.S.

Goldfine AB, Fonseca V, Jablonski KA, et al. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152: 346-357, 2010.

Intensive Lifestyle Intervention Reduces Risk Factors for Cardiovascular Disease in People with Type 2 Diabetes: In a major ongoing NIDDK-supported clinical study, researchers showed that after 4 years, an intensive lifestyle intervention (ILI) program reduces cardiovascular disease (CVD) risk factors in overweight and obese people with type 2 diabetes. Although the short-term benefits of lifestyle interventions to achieve weight control are well understood, long-term effects of intervention on

blood glucose (glycemic) control and CVD risk factors have not been extensively studied. The nationwide Look AHEAD (Action for Health in Diabetes) trial is investigating the long-term health effects of a sustained ILI program, as compared to the current diabetes support and education (DSE) strategies, on improving cardiovascular outcomes. More than 5,000 overweight or obese participants between the ages of 45 and 76, with type 2 diabetes, were randomly assigned to either the ILI or DSE strategy. Individuals in the DSE group were invited to three group sessions per year that provided information conveying the importance of controlled diet, nutrition, and physical activity, as well as social support sessions. In addition to the information provided to the DSE group, individuals in the ILI group received defined, reduced caloric intake and physical exercise goals, frequent weighing, and specific behavior modification instruction in self-monitoring (such as maintaining a diet and exercise diary), problem solving, and goal setting. The participants receiving ILI also had more frequent group meetings, as well as regular one-on-one lifestyle counseling.

The Look AHEAD study had previously reported beneficial health effects after 1 year, and the investigators have now found that participants in both groups showed positive changes in their health over 4 years. On average, across all 4 years, participants in the ILI group lost significantly more weight than those in the DSE group (6.2 percent vs. 0.9 percent reduction). ILI group members also experienced improved fitness, glucose control, blood pressure, and HDL (good) cholesterol. Both groups showed reductions in LDL (bad) cholesterol, but the reductions were larger in the DSE group because of their greater use of cholesterol-lowering medications.

A vast majority of participants remain in the study after 4 years, and the trial is planned to continue for up to 13.5 years. These findings reveal a strong link between intensive lifestyle intervention and improvements in CVD risk factors associated with overweight, obesity, and diabetes. Longer-term results from this ongoing trial will determine whether these effects can be sustained and whether they will ultimately lead to reduced incidence of illness and death from CVD, as well as other health benefits.

The Look AHEAD Research Group. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med 170: 1566-1575, 2010.

New Insights into Treating People with Type 2 Diabetes and a High Risk of Heart Disease:

Results from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial are providing important evidence to help guide treatment recommendations for adults with type 2 diabetes who have had a heart attack or stroke or who are otherwise at high risk for heart disease. ACCORD investigators studied over 10,000 adults who had type 2 diabetes for an average of 10 years and were at especially high risk for cardiovascular events. All participants were enrolled in the ACCORD blood glucose treatment clinical trial; participants were also enrolled in either the blood pressure trial or the lipid trial.

Results of the ACCORD blood glucose trial were reported in 2008 and showed that intensively lowering blood glucose to near-normal levels brought a higher risk of death for participants than standard blood glucose control. More recently, results of the lipid and blood pressure trials were announced. In the blood pressure trial, researchers randomly assigned participants with elevated blood pressure to a target systolic blood pressure—the top blood pressure number—of either less than 140 (the standard group) or a "normal" level of less than 120 (the intensive group). The study found that lowering blood pressure to normal levels did not significantly reduce the risk of cardiovascular events overall, although it may reduce the risk of stroke. However, this treatment strategy was associated with more complications, such as abnormally low blood pressure. In addition, some laboratory measures of kidney function were worse in the intensive therapy group, but there was no difference in the rates of kidney failure. In the lipid trial, researchers compared two groups. One group received a fibrate medication, which lowers triglycerides and raises HDL (good) cholesterol, while the other group did not. Both groups received a statin medication, which lowers LDL (bad) cholesterol. Combination therapy of statin and fibrate medications appeared to be safe, but did not lower the risk of heart attack, stroke, or death from heart disease more than the statin alone.

In addition to examining the effects of the different therapies on macrovascular (large vessel) damage to the heart, ACCORD researchers also studied the effect of these therapies on microvascular (small vessel) damage to organs and tissues. Intensive blood glucose control was found to reduce some indicators of eye, nerve, and kidney disease compared to standard control, but the intensive and standard control groups did not differ in the rate of progression to kidney failure, nerve disease, and major vision loss. The results are consistent with findings from the ACCORD Eye Study, which found that, in a subset of participants, intensive blood glucose control reduced progression of a form of eye disease called diabetic retinopathy. The scientists measured less severe eye damage in the Eye Study and saw a benefit, but there was no benefit observed with respect to more severe damage—major vision loss—in the full trial. The Eye Study also found that adding a fibrate drug to statin therapy for control of blood lipids reduced progression of diabetic retinopathy. However, intensive blood pressure control provided no additional benefit compared with standard blood pressure control.

Although the ACCORD finding of increased mortality risk outweighed the benefits of near-normal glucose control in the participants of this trial, it remains possible that more intensive blood glucose control may be more beneficial earlier in the course of type 2 diabetes than was studied in ACCORD. Optimal therapy for older patients newly diagnosed with diabetes or without complications will have to be tested in future research. The ACCORD results are helping health care providers tailor therapy for their patients with type 2 diabetes, by considering the risks and benefits of different treatment approaches for each patient.

ACCORD Study Group, Ginsberg HN, Elam MB, et al. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med 362: 1563-1574, 2010.

ACCORD Study Group, Cushman WC, Evans GW, et al. Effects of intensive blood-pressure control in type 2 diabetes mellitus. N Engl J Med 362: 1575-1585, 2010.

Ismail-Beigi F, Craven T, Banerji MA, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet 376: 419-430, 2010.

ACCORD Study Group; ACCORD Eye Study Group, Chew EY, et al. Effects of medical therapies on retinopathy progression in type 2 diabetes. N Engl J Med 363: 233-244, 2010.

REGULATORS OF METABOLISM IN HEALTH AND DISEASE

Molecular Insights Could Lead to Improved Type 2 Diabetes Drugs: Researchers have identified the molecular mechanism by which the master regulator of fat cell biology and development, a protein called PPAR-gamma, regulates insulin sensitivity. A hallmark of type 2 diabetes is that cells become resistant, or less sensitive, to the action of insulin, a hormone that promotes the uptake of glucose from the bloodstream into the cells. Some type 2 diabetes drugs have been developed to target PPAR-gamma; the drugs make the body more sensitive to insulin, but come with unwanted side effects, such as weight gain and an increased risk of heart failure. In this study, scientists discovered that a specific chemical modification (phosphorylation) to PPAR-gamma leads to the abnormal regulation of a number of genes related to obesity and insulin sensitivity in mice. They also found that the diabetes drugs block this modification in people with type 2 diabetes, thus countering insulin resistance. The drugs also broadly stimulate PPAR-gamma, which may be responsible for the negative side effects. The research suggests that a new generation of type 2 diabetes drugs could be designed to block the chemical modification only, without broadly stimulating PPAR-gamma, to improve insulin sensitivity without the unwanted side effects.

Choi JH, Banks AS, Estall JL, et al. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPAR gamma by Cdk5. Nature 466: 451-456, 2010.

Understanding How Cells Relieve Stress—Implications for Diabetes: Scientists uncovered how the Wolfram syndrome 1 (WFS1) protein protects cells, including pancreatic beta cells, from an uncontrolled response to a type of cellular stress. Mutations in the gene encoding WFS1 cause Wolfram syndrome, a genetic form of diabetes also associated with optic atrophy, neurodegeneration, and psychiatric illness. Diabetes resulting from Wolfram syndrome is characterized by non-immune mediated loss of the insulin-producing beta cells. Beta cell death in

Wolfram syndrome has been suggested to result from an uncontrolled response to a type of stress in a cellular component called the endoplasmic reticulum (ER). One of the critical processes that occur in the ER is the process of "protein folding" by which a protein acquires its mature structure. Stress on the ER, whether caused by protein misfolding or other various stimuli, can lead to detrimental consequences. In response to this stress, the cell instigates a response which ultimately leads to the production of proteins that relieve the stress. This response, however, must be mitigated once the stress is alleviated because hyperactivation of the stress response can lead to cell death.

WFS1 had previously been shown to mitigate the ER stress response in cells, but in a new study, scientists revealed the specific role WFS1 plays in the stress response. Using rodent and human cells, the scientists determined that WFS1 associates with another protein—ATF6alpha—a key regulator of the response to ER stress. They found that WFS1 suppressed ATF6alpha activity by recruiting it to a protein degradation complex, thus lowering levels of ATF6alpha and keeping the stress response restricted. In response to a stress signal, however, the interaction between WFS1 and ATF6alpha was disrupted, freeing ATF6alpha to turn on genes whose protein products function to relieve the ER stress. When the scientists depleted WFS1 from laboratory-grown cells, however, they observed that ATF6alpha levels were increased, and the stress response was chronically hyperactivated. Moreover, beta cells from mice genetically engineered to lack WFS1 and other types of cells (lymphocytes) from patients with Wolfram syndrome similarly exhibited abnormally high amounts of ATF6alpha.

These results indicate that WFS1, by working through ATF6alpha, has a critical role in regulating the ER stress response and in preventing cells from dysfunction and cell death caused by a hyperactive stress response. Interestingly, variants in the gene encoding WFS1 are also associated with type 2 diabetes. This research suggests that WFS1 could be a key target for strategies to treat or prevent diseases related to ER stress, including diabetes.

Fonseca SG, Ishigaki S, Oslowski CM, et al. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. J Clin Invest 120: 744-755, 2010.

Stoking the Fat Furnace with SIRT3: Researchers have uncovered a new mechanism regulating how cells burn fat. Fat tissue holds the body's major energy reserve. When the body needs to tap into this reserve, such as during a fast, fat molecules are mobilized to other organs and tissues. There, mitochondria—the "powerhouses" of the cell—can switch from burning glucose, the primary cellular fuel, to burning fat for energy. New research suggests that a protein called SIRT3 regulates this important switchover in metabolism. In a series of experiments, scientists compared metabolism between normal mice and mice genetically engineered to lack SIRT3, under both fed and fasted conditions. They found that during a fast, mice lacking SIRT3 had incomplete fat-burning in their livers, resulting in abnormally high levels of fat intermediates and triglycerides. Moreover, while mice with and without SIRT3 both appeared normal in the fed state, mice lacking SIRT3 showed symptoms of fat-burning disorders when challenged with specific metabolic stresses. For example, when fasted, these mice produced less energy in their livers and had low tolerance to cold. SIRT3 activates other proteins, such as metabolic enzymes, by removing specific chemical modifications called acetyl groups. Molecular experiments revealed that a key enzyme in fat-burning is regulated by these modifications and needs SIRT3 in order to be activated during a fast. Because SIRT3 itself depends on a molecule whose levels reflect the cell's metabolic state, the scientists hypothesize that SIRT3 acts as a "metabolic sensor," enabling cells to quickly switch to fat-burning in response to energy needs. Defects in fat-burning are associated with diabetes and other metabolic disorders. Researchers can now explore the possible pathogenic role of SIRT3 in metabolic disorders and the potential therapeutic value of boosting SIRT3 activity.

Hirschey MD, Shimazu T, Goetzman E, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464: 121-125, 2010.

Candidate Coordinator for Inflammation and Metabolic Diseases: A molecule that helps the body fight viral infections may also play an important role in metabolic diseases. Research over the past decade demonstrated that inflammation, normally one of the body's defenses against invading microbes, can contribute to very different processes—the development

and progression of chronic metabolic conditions such as obesity and diabetes. How and why this happens is being actively investigated so that new therapeutic and preventive approaches may be developed. Contemplating how inflammation might connect the seemingly diverse conditions of infection and metabolic disease, scientists have speculated that perhaps a molecule(s) governing infection-triggered inflammation is also stimulated when the body has excess nutrients, as in obesity. New research suggests that a molecule called double-stranded RNA-dependent protein kinase, or PKR, could be a candidate. Known previously for its important role in detecting viral invasion and orchestrating antiviral responses in a cell, PKR is also associated with an inflammatory signaling pathway that has been implicated in insulin resistance—a condition associated with type 2 diabetes and obesity.

To determine whether PKR coordinates activities that disrupt metabolism, scientists studied PKR in mouse models and cells. These studies revealed that PKR is indeed activated in response to nutrient excess and other metabolic stresses and can inhibit a key component of insulin signaling, both directly and by activating the inflammatory pathway. Moreover, in experiments with overfeeding, mice genetically engineered to lack PKR activity had less body fat, inflammation, and insulin resistance than mice with normal PKR after a number of weeks on a high-fat diet. Notably, when the mice were fed a regular diet, those lacking PKR activity were still more insulin sensitive than normal mice. These results suggest that PKR may coordinate both increased inflammation and metabolic malfunction in response to nutrient excess and other metabolic stresses. Understanding the regulation of PKR may also provide some insights into metabolic problems associated with viral infections. PKR may thus emerge as a therapeutic target in chronic metabolic diseases.

Nakamura T, Furuhashi M, Li P, et al. Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell 140: 338-348, 2010.

Discovery of a Pancreas-Specific Clock Associated with Diabetes: Scientists discovered that the pancreas has its own molecular clock, and that clock defects trigger onset of diabetes in a mouse model. In animals and humans, the circadian clock regulates many behaviors and bodily processes—including sleep/wake cycles, changes in blood pressure, and body temperature fluctuations—to harmonize these activities with daily, rhythmic changes in the environment, most notably day/night cycles. Metabolism is also rhythmically controlled, and disruption of these cycles is associated with type 2 diabetes. Researchers examined whether the circadian clock specifically played a role in regulating insulin release from pancreatic islets in response to glucose. The scientists found that, in a mouse model, the islets had their own molecular clock, distinct from the primary circadian clock that resides in the brain. When the researchers generally disrupted the circadian clock in mice, the animals had impaired tolerance to glucose, reduced insulin secretion, and smaller islets, suggesting that the pancreatic clock directly regulates insulin production. When the clock disruption was limited to the pancreas alone, the animals had elevated blood glucose levels and impaired insulin secretion in response to glucose, resulting in the animals developing diabetes. The research has identified a pancreas-specific clock—distinct from the body's overall circadian clock—that plays an important role in regulating metabolism. Targeting the proteins involved in regulating this clock is a possible strategy for treating diabetes.

Marcheva B, Ramsey KM, Buhr ED, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 466: 627-631, 2010.

TREATING SEVERE INSULIN RESISTANCE

Drug Combination Neutralizes Severe Insulin Resistance in a Rare Disease: A combination of therapeutic drugs has been found to reverse the devastating effects of "type B" insulin resistance. When the body becomes impaired in its ability to use insulin to regulate glucose uptake by cells, a variety of metabolic diseases can arise. One relatively rare but serious, often fatal, disease is type B insulin resistance. In this autoimmune condition, the body launches a misguided immune attack against insulin's protein partner, known as the insulin receptor, which resides on the surface of cells and effects the action of insulin. In people with type B insulin resistance, the body inappropriately produces antibodies that prevent interaction of insulin with its receptor, blocking insulin's ability to communicate with target cells. The resulting insulin

signal interference leads to blood glucose imbalance and numerous negative physiological effects, such as weight loss, excess testosterone production, and unusual skin discoloration (known as acanthosis nigricans). While the condition can affect anyone, type B insulin resistance occurs predominantly in African American women. Previously, therapeutic strategies to restore the body's ability to regulate glucose in patients with type B insulin resistance had only been modestly successful.

Recently, scientists in NIDDK's Intramural Research Program, in collaboration with other researchers, identified a combination of drugs that was remarkably effective in reversing the physiological effects of insulin resistance. This drug combination was designed to remove existing circulating antibodies, as well as to prevent the production of new antibodies, and included steroids and other immunosuppressive drugs, as well as a drug called rituximab, which causes the depletion of antibody-producing cells. Due to the rarity of this disease, only a few patients were treated in this study. However, the results were clear: all seven people with type B insulin resistance who received treatment were in remission, generally within 1 year. Importantly, there were minimal side effects from the treatment regimen. These findings not only establish a promising new treatment strategy for those suffering from type B insulin resistance, but also provide a framework for designing effective therapies for other autoimmune disorders.

Malek R, Chong AY, Lupsa BC, et al. Treatment of type B insulin resistance: a novel approach to reduce insulin receptor autoantibodies. J Clin Endocrinol Metab 95: 3641-3647, 2010.

CYSTIC FIBROSIS RESEARCH

Potential Avenues for Treating Cystic Fibrosis—Partially Restoring the Function of One Protein by Inhibiting the Function of Others: New research suggests it may one day be possible to treat many people with cystic fibrosis (CF) by inhibiting an enzyme called histone deacetylase 7 (HDAC7) and/or by interfering with the cell's protein "quality control" machinery. Most people with CF have a version of the CF gene that generates a misfolded form of CFTR, the most functionally significant CF protein. Because it is not folded correctly, this variant of CFTR does not reach its normal location at the cell surface, where it is needed to transport salts and keep the airways of the lungs properly hydrated. Researchers recently found that inhibiting the action of the HDAC7 protein allows some of the variant CFTR to fold so that it can reach the cell surface and function. These experiments were performed in cultured human lung cells, so it is not yet clear whether a similar approach can safely be taken to treat patients with CF. However, one of the compounds used in this study to inhibit HDAC7 already has been approved by the U.S. Food and Drug Administration for treatment of cutaneous T cell lymphoma, a form of cancer. It is not certain precisely how HDAC7 inhibition affects CFTR, but it is likely to be an indirect effect, possibly involving several other genes and proteins. Further research is needed to address this question. These results suggest that inhibiting HDAC proteins may be a viable approach for treating some people with CF, and possibly for treating people with other diseases related to protein folding problems.

Although strategies, such as inhibiting HDAC7, that increase the amount of CFTR reaching the cell surface are a promising approach to treatment, they are potentially hampered by the cell's protein quality control machinery, which both prevents abnormal proteins from reaching the cell surface and removes them from the surface if they do arrive—even if the abnormal proteins are partly functional or could be made functional with therapeutics. New research has helped clarify the set of proteins that are involved in recognizing and removing abnormal or misfolded CFTR from the cell surface, and targeting it to be degraded within the cell. Working with cultured human cells expressing the same misfolded CFTR variant referred to above, researchers used a molecular tool called small interfering RNAs (siRNAs) selectively to eliminate specific proteins involved in the cell's quality control process. By interfering with one of these proteins at a time, they were able to identify those that are integral to removing misfolded CFTR from the cell surface. Thus, it may one day be possible to increase the amount of functioning CFTR reaching the surface of the cell through the use of an inhibitor of HDAC7 or a related approach, while at the same time, inhibiting the cell's quality control machinery may increase the time that CFTR spends on the cell surface, where it functions as an ion transporter.

Hutt DM, Herman D, Rodrigues APC, et al. Reduced histone deacetylase 7 activity restores function to misfolded CFTR in cystic fibrosis. Nat Chem Biol 6: 25-33, 2010.

Okiyoneda T, Barrière H, Bagdány M, et al. Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science 329: 805-810, 2010.

Ferreting Out the Complex Biology of Cystic Fibrosis: Researchers have recently developed a new animal model of CF that may help answer important questions about the biology of the disease and advance the development and testing of new candidate CF therapies. Although chronic, damaging lung infections are the most frequent cause of death for people with CF, the disease also has a serious impact on the pancreas, liver, intestine, gallbladder, sweat glands, and male reproductive tract. Digestive consequences of the disease in the intestine and pancreas, for example, are of enormous significance for children with the disease because they severely interfere with the ability of the body to absorb key nutrients and fuel proper growth and development. Mouse and pig models of CF have helped clarify what goes wrong in the many organ systems affected by the disease, but their utility has been limited by differences between these animals and humans in organs and tissues and the effects of the disease. The ferret respiratory system is markedly more like humans' than is the airway of mice. In particular, the distribution of the CFTR protein is quite similar in the ferret and human airways.

For this reason, researchers generated a model of CF by eliminating the ferret *CFTR* gene in test animals, and examined the physiological consequences. They found that the effect on the ferret respiratory tract was much like what is seen in humans with CF, but the effect on the ferret digestive tract was more severe than is seen in people with this disease. To facilitate study of other aspects of the disease, the researchers found two ways of getting around the severe digestive complications of ferret CF: using medications that help alleviate these digestive consequences, and creating another line of animals in which CFTR is functional in the intestinal cells but not in other organs. Both approaches showed promise in yielding animals that may be of great potential value in terms of understanding the effect of CF on human organ systems, in particular the respiratory tract, and for helping to develop and test potential new therapeutic approaches to treating the disease.

Sun X, Sui H, Fisher JT, et al. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. J Clin Invest 120: 3149-3160, 2010.

Dr. Muneesh Tewari and Dr. Martin T. Zanni: NIDDK-Supported Scientists Receive Presidential Award

On November 5, 2010, President Barack Obama recognized 85 U.S. scientists, including two supported by NIDDK, with the Presidential Early Career Award for Scientists and Engineers (PECASE; *www.whitehouse.gov/administration/eop/ostp/pressroom/11052010*).

PECASE is the most prestigious award given in the U.S. to scientists at the outset of their independent research careers. The 2009 recipients were honored at a White House ceremony with the President in December 2010.

PECASE is awarded annually to scientists and engineers who, while early in their research careers, have demonstrated the pursuit of innovative research and outstanding scientific leadership. Among the 2009 recipients are Muneesh Tewari, M.D., Ph.D. and Martin T. Zanni, Ph.D., both NIDDK extramural grantees.

In addition to Drs. Tewari and Zanni, 18 other NIH-supported scientists received the award for their research achievements. The NIH has now funded 173 PECASE recipients since the award's inception in 1996. A list of NIH scientists who have received this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

Investigations into a Potentially New Type of Hormone

Muneesh Tewari, M.D., Ph.D.

Dr. Tewari, an oncologist and cancer researcher at the Fred Hutchinson Cancer Research Center in Seattle, received a 2009 PECASE award for his innovative work on the release of RNA from cancer cells that may lead to early cancer detection with the potential for novel therapies. The conventional norms of the mammalian endocrine system do not generally consider RNA molecules as a class of hormones. Hormones are small molecules that are secreted into the blood and affect the function of distant tissues and organs. Dr. Tewari points to several facts to propose that RNA molecules may also function as hormones in mammals. First, RNA molecules have been demonstrated to function as hormones in plants. Second, in some animal species (*e.g.*, worms and flies) RNA has been shown to spread from one site to another. And third, one class of RNAs, known as microRNAs, are abundantly present in the blood of healthy people, and specific microRNAs accumulate in states such as cancer, diabetes, and other diseases. To lay the foundation of whether microRNAs can act as a type of hormone in mammals, Dr. Tewari is studying microRNAs secreted into the blood by cancer cells to determine whether they are taken up by and influence distant organs and tissues. Establishing that RNA molecules in the blood can act as hormones could lead to better methods of diagnosing and treating a variety of human diseases.

Developing Cutting-Edge Technologies To Study Health and Disease

Martin T. Zanni, Ph.D.

Dr. Zanni, the Meloche-Bascom Professor of Chemistry at the University of Wisconsin-Madison, received a 2009 PECASE award for his research developing novel spectroscopic methodologies to study the molecular mechanisms by which biomolecules cause disease. He specializes in the development of two-dimensional infrared (2D-IR) spectroscopy and its application to problems in biophysics and human health. He has broken new ground in understanding infectious diseases with a novel

discovery about the structure of the influenza virus's M2 protein, a major target of anti-influenza drugs. Dr. Zanni is currently using 2D-IR spectroscopy to uncover key details about amyloid toxicity with implications for the treatment of type 2 diabetes. A feature of type 2 diabetes is the presence of amyloid fibers in the pancreas. These fibers are composed of the human islet amyloid polypeptide (hIAPP), and many *in vitro* and *in vivo* studies have linked them to the disease. However, the mechanism by which hIAPP promotes the death of the insulin-producing cells is not understood. A growing body of evidence points to special molecular species of hIAPP interacting with the cell membrane as the cause of cell death rather than the fibers themselves. Using 2D-IR spectroscopy, Dr. Zanni is characterizing these molecular species and their interaction with the cell membrane to better understand their contribution to the development of type 2 diabetes.

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What's Old Is New Again: Targeting Inflammation To Treat Diabetes

Researchers have recently made an important clinical advance that has its origins in surprising observations about type 2 diabetes that began to accumulate well over a century ago. At that time, insulin had not yet been discovered, and there was no effective way to treat any form of diabetes. Indeed, aspirin had not yet been invented, although earlier forms of the drug—called salicylates—were known to reduce the pain, fever, and swelling of inflammation. In reports published in 1876 and 1901, clinicians found that high-dose salicylates partly alleviated diabetes as measured by the earliest known biomarker of the disease—glucose in the urine. Unfortunately, the approach was impractical, because high doses of salicylates have significant side effects and, in particular, are damaging to the stomach. Thus, for many decades the result remained a puzzle to researchers, to the extent that it was remembered at all. Why would anti-inflammatory medications like salicylates relieve diabetes when neither pain, nor any of the other hallmarks of inflammation known since antiquity—fever, swelling, and redness—are intrinsic to diabetes?

Inflammation is essentially an "SOS" signal sent by cells called macrophages to other components of the immune system. Macrophages send this signal in response to injury or infection, and gradually turn the signal off as the wound or infection heals. By the middle of the 20th century, scientists were beginning to piece together some of the molecular details of the inflammatory process. For example, the serum concentrations of a variety of proteins known as "acute phase proteins" were found to rise or fall in conjunction with inflammation. The first clue to understanding the surprising efficacy of salicylates came in the 1950s, when some of these acute phase proteins were found to be elevated in diabetes. At the time, the significance of this finding was not widely appreciated.

The connection between diabetes and inflammation did not begin to come into sharper focus until pioneering NIDDK-funded work in the 1990s found that an acute phase protein called TNF-alpha was produced in adipose (fat) tissues of obese mice. More provocatively, the researchers found that producing excess TNF-alpha in non-obese mice could induce insulin resistance, a condition that can lead to type 2 diabetes and that is also a hallmark of the disease. The researchers also found that deleting the TNF-alpha gene, or the gene of another protein that is required for its activity, actually protected mice from type 2 diabetes. These results suggested an answer to a question that had vexed the field for a long time—what is it about excess adipose tissue that promotes diabetes? The data raised the possibility that the immune system may play a role in insulin resistance, even in the absence of infection.

Indeed, NIDDK-supported researchers found that macrophages accumulate in the adipose tissue of obese rodents, as well as humans. But what is it about obesity that attracts macrophages to an uninjured, uninfected part of the body? The answer has to do with a group of proteins called Toll-like receptors (TLRs), which recognize foreign material in the body, such as molecules on the outside of invading bacteria, and in response trigger a powerful inflammatory signal. NIDDK-supported researchers found, in 2001, that one of these proteins, TLR4, which is produced both by macrophages and fat cells, is activated not only by bacteria, but also by high levels of free fatty acids, an important form

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of unstored fat in the body. Researchers found that mice lacking TLR4 are protected from insulin resistance caused by a high-fat diet, showing that signaling through TLR4 plays a key role in the central pathological process that leads to type 2 diabetes. Thus, it is now believed that the presence of high levels of free fatty acids in adipose tissue triggers TLR4 on fat cells to induce production of inflammatory signals, which draws macrophages to the tissue. Macrophage-produced TLR4 also responds to the fatty acids, ramping up the inflammatory process.

A parallel line of inquiry was clarifying the role of salicylates. These medicines, it turns out, can impinge on the inflammatory process in two distinct ways. First, they can inhibit the activity of an enzyme known as cyclooxygenase, which has a key role in inducing many of the classical symptoms of inflammation, including fever and pain. Second, they can interrupt an enzyme called I-kappa-B kinase beta (IKK-beta), which has a key role in transmitting the inflammatory signal stimulated by TNF-alpha, and which turns out to be relevant to diabetes. NIDDK-supported research showed that inhibiting IKK-beta can reverse insulin resistance in rodent models of type 2 diabetes, and that stimulating IKK-beta can trigger insulin resistance. These results help explain how salicylates might counteract type 2 diabetes. Additionally, IKK-beta is less sensitive to aspirin and other salicylates than is cyclooxygenase, a finding that may help explain why aspirin and related pain relievers, when given in moderate doses, do not alleviate diabetes. Indeed, as suggested by the clinical data on salicylates from the 19th century, high-dose salicylates were found to reverse insulin resistance in obese mice.

This basic research set the stage for a modern re-test of salicylates for the treatment of type 2 diabetes. In 2002, NIDDK-supported researchers reported the metabolic effect of high-dose aspirin on seven people with type 2 diabetes. The treatment significantly improved insulin sensitivity, and lowered levels of blood glucose, cholesterol, and other fats, without affecting body weight. As with the salicylates tested more than a century earlier, the significant side effects of high-dose aspirin, which can include gastrointestinal ulcers and bleeding, especially in the stomach, make it unsuitable as a long-term therapy for diabetes. However, the result demonstrated that IKK-beta inhibition might be a pharmacologically effective approach for treating type 2 diabetes, if a method could be found to do so safely.

It turned out that a form of salicylate was already known which seemed to have suitable properties. Salsalate, a pain medication used for decades as a treatment for rheumatoid arthritis, is notable for lacking many of the high-dose side effects of aspirin, and is available as an inexpensive, generic prescription drug. Small, preliminary trials of salsalate indicated that it may be effective as a treatment for type 2 diabetes. The NIDDK established the Targeting INflammation Using SALsalate in Type 2 Diabetes (TINSAL-T2D) clinical study to more rigorously test the approach. In the trial, 108 participants who had recently been diagnosed with type 2 diabetes were randomly assigned to receive either placebo or one of three different doses of salsalate, for 14 weeks. Researchers tracked changes in participants' hemoglobin A1c (HbA1c) levels—used to track blood glucose control—as well as other metabolic measures, and also monitored for side effects.

As reported in 2010, those participants receiving any of the three doses of salsalate experienced a significant reduction in HbA1c level compared to those in the placebo group, indicating that salsalate was effective in improving blood glucose control. Those who took salsalate also saw improvements in their blood levels of triglycerides (a type of fat), suggesting the salsalate might help lower their risk of cardiovascular disease, which has also been linked to inflammation. Although there were

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not many side effects, one was that salsalate was associated with an increase in the concentration of a protein called albumin in the urine. Urine albumin is a marker of kidney disease, which is an important complication of diabetes, so this observation could indicate a problem with salsalate as a type 2 diabetes treatment. In contrast, another marker of kidney function, cystatin C, did not change in any of the groups over the course of the trial, and thus it is unclear whether or not salsalate might harm kidney function. This question will be examined carefully in future tests. In addition, participants receiving salsalate were more likely than those receiving placebo to experience mild episodes of low blood glucose. This side effect was only observed in patients who were also taking a diabetes medication known as sulfonylurea, suggesting the two drugs may not be suitable for use in tandem.

The blood glucose level finding is also an indication that salsalate is lowering blood glucose, because diabetes is essentially the state of chronically elevated blood glucose.

Based on these encouraging results, an expanded study of safety and efficacy is now being conducted in a larger number of patients, with a longer duration of treatment. Designated "TINSAL-T2D-Stage II," the trial is recruiting participants for a 48-week treatment course with salsalate or placebo. If the approach is successful, this research could offer a new treatment option for diabetes using an inexpensive medication that has been found to be safe and effective over decades of use in patients with arthritis. And the use of anti-inflammatory agents for diabetes, first tested more than 130 years ago, may soon reach fruition as a therapeutic approach.

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Gestational Diabetes and the Health of Generations

Answers about the causes of human obesity and type 2 diabetes may lie not only with genetic and environmental factors, but also with factors encountered while in the womb. With the hope of understanding and potentially preventing chronic disease, researchers have been studying the impact of maternal diabetes, particularly gestational diabetes mellitus (GDM)—a form of diabetes similar to type 2 diabetes, but unique to pregnancy—on the health of women and their offspring. From these studies, we now know that GDM is not simply a temporary condition with potentially acute consequences during late pregnancy and delivery. Rather, diabetes during pregnancy appears to increase long-term metabolic health risks for both mother and child. Moreover, results from a major clinical study of maternal blood glucose levels and pregnancy outcomes have led clinicians to reconsider current criteria for diagnosing and treating GDM. These discoveries are providing new opportunities to improve the health of women and their families.

A form of diabetes that is first diagnosed during pregnancy, GDM is estimated to affect about 7 percent of all U.S. pregnancies—about 200,000 each year.[1] While it usually resolves after delivery, research has shown that women who have been diagnosed with GDM are at significantly increased risk of having it again during future pregnancies and/or getting diabetes, primarily type 2 diabetes, later in life. GDM also increases the risk of complications for mother and child during pregnancy and delivery. While the cause of GDM is not fully known, it is thought that hormonal changes during pregnancy contribute to its development.

As early as 1952, researchers recognized that a pregnant woman's metabolic health could have an impact on the developing fetus. A Danish scientist, Dr. Jørgen Pedersen, proposed that elevated blood glucose levels in the mother lead to elevated glucose levels in the infant, who then responds by increasing insulin production, which promotes storage of energy as fat—even to excess. In subsequent years, this hypothesis was further refined as more was learned about the contributors to an over-nutritive intrauterine environment and its effects on a developing fetus, a concept known as "fuel-mediated teratogenesis."

In addition to the immediate consequences of maternal diabetes on offspring, scientists were curious about the impact that these early exposures in the intrauterine environment might have long-term. Scientists in NIDDK's Intramural Research Program were among the first to shed light on these questions. Working with the Pima Indians in Phoenix, Arizona, a population having one of the highest rates of obesity and type 2 diabetes in the world, NIDDK researchers have been able to learn a great deal about the genetic and environmental factors influencing development of these conditions. In 1983, NIDDK researchers published the results of a study of children born to Pima Indian women with and without diabetes during pregnancy. These included both women who developed GDM and women who already had type 2 diabetes prior to pregnancy. The results clearly linked maternal diabetes during pregnancy with higher rates of obesity in offspring during their childhood and teen years. Subsequently, in the late 1980s, the research team found that offspring of Pima Indian women who had diabetes during pregnancy were much more likely to develop type 2 diabetes as young adults compared to children of women who did not have diabetes during pregnancy. Another key study was published in 2000, in which NIDDK researchers examined Pima Indian mothers with type 2 diabetes who had multiple pregnancies—some before they developed the disease, and some after. The children born from a diabetic pregnancy had a higher chance of being obese or developing diabetes than those—from the

same mother—born from pregnancies before the mother developed type 2 diabetes. This study allowed the scientists to zero in on the effect of the intrauterine environment *per se*—because the babies were born to the same mother, genetic factors were not playing a role. Importantly, these and other studies in the Pima Indians enabled researchers to determine that the intrauterine environment exerts an effect on the development of obesity and diabetes in youth that is distinct from genetic and environmental contributors.

Do the findings in the Pima Indian population hold true in more diverse populations in the U.S.? Answers have emerged from the SEARCH for Diabetes in Youth (SEARCH) study. SEARCH is examining the prevalence and incidence of diabetes in racially and ethnically diverse children and youth, as well as elucidating factors that contribute to development of diabetes before the age of 20. SEARCH researchers have found that maternal diabetes appears to accelerate onset of type 2 diabetes in youth. Among study participants with type 2 diabetes, those whose mothers had diabetes during pregnancy were diagnosed at a younger age than those whose mothers were diabetes free. A related study using SEARCH data found that youth with type 2 diabetes were much more likely to have been exposed to diabetes or obesity while *in utero* than youth without diabetes. These results indicate a universal effect of intrauterine exposure that operates, in addition to factors such as genetics and race/ethnicity, to increase lifetime risk for developing type 2 diabetes.

Spurred by these studies in people, researchers have also studied the impact of maternal diabetes in animals. Together, the studies in people and animals have led to the current model that "diabetes begets diabetes." A key aspect of this model is the threat of a vicious cycle, in which daughters of diabetic pregnancies develop diabetes themselves prior to or at the time of pregnancy, perpetuating the impact of diabetes on offspring via the intrauterine environment. The urgency of these findings is underscored by findings in the last several years among the Pima Indians, demonstrating an increased incidence of diabetes complications at an earlier age among people diagnosed with type 2 diabetes in youth. As there are an increasing number of people developing diabetes and obesity at younger ages, it will be important to try and break this vicious cycle by preventing or mitigating the effects of diabetes and obesity during child-bearing years and pregnancy.

The criteria for GDM were originally established based on risk to the mother of subsequently developing type 2 diabetes. Therefore, an important question has been whether GDM—or even hyperglycemia at levels below traditional GDM criteria—has adverse pregnancy outcomes for mother and child. The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, led by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development with co-support from NIDDK, sought to determine whether blood glucose levels that are lower than those used to diagnose GDM have acute consequences on pregnancy outcomes. By examining blood glucose levels and pregnancy outcomes in over 23,000 women around the world, the HAPO study demonstrated that there is a continuum of risk according to glycemia at levels below those currently used to diagnose GDM. In the study, the higher a pregnant woman's blood glucose levels following an oral glucose tolerance test, the more likely she was to deliver a high-birthweight baby, and the more likely the baby was to have signs of hyperinsulinemia (excessive levels of circulating insulin in the blood). Elevated blood glucose also raised the risk for extremely serious problems for the mother and child, such as preeclampsia in the mother and shoulder dystocia for the newborn during birth. Most significantly, there was no clear threshold level at which these outcomes began—that is, there is apparently no "safe" level of hyperglycemia. As a result of the HAPO study and other clinical studies, an international panel has recommended new criteria

for diagnosing GDM. These criteria would potentially increase the burden of gestational diabetes from 7 to nearly 18 percent of pregnancies, and have now been adopted by the American Diabetes Association.

Scientists are still striving to understand the biological underpinnings of the long-term health effects of diabetes and obesity during pregnancy. Studies in humans, as well as in animal models, are under way to try to understand the mechanisms by which the intrauterine environment determines disease later in life. Research indicates that obesity and hyperglycemia during pregnancy are associated with epigenetic changes, which are changes in the regulation of gene activity and expression (whether genes are turned on or off) that are not dependent on the sequence of the DNA. While scientists work to understand the mechanisms underlying "metabolic imprinting," continued research could help determine how best to protect the growing fetus from an adverse metabolic milieu, and break the vicious cycle of trans-generational transmission of metabolic disease.

While many questions remain, the work of numerous researchers over several decades revealed that diabetes during pregnancy has long-term effects on the metabolic health of mothers and their children.

The National Diabetes Education Program (NDEP), which is co-led by NIDDK and the Centers for Disease Control and Prevention (CDC), is a platform for disseminating evidence-based information about prevention and treatment of diabetes and its complications. In partnership with the NIH Office of Research on Women's Health, NDEP is translating what is known about GDM, through a campaign called "It's Never Too Early to Prevent Diabetes." This campaign is reaching out to women and their health care providers to raise awareness that women with a history of GDM are at increased risk of diabetes, and their children are at increased risk of type 2 diabetes and obesity. It also imparts a hopeful message, based on the results of the NIDDK-led Diabetes Prevention Program clinical trial, namely that type 2 diabetes can be prevented or delayed in women with a history of GDM through an intensive lifestyle intervention of diet and exercise or through use of the diabetes drug metformin. Continued research on this front will help break the vicious cycle and help to avert the serious health problems of diabetes and obesity in future generations.

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[1] *American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 27: S88-S90, 2004.*

Chronic Disease and the Regulation of Inflammation

Dr. Christopher K. Glass

Dr. Christopher K. Glass holds joint appointments in the Department of Medicine and in the Department of Cellular and Molecular Medicine at the University of California, San Diego (UCSD). Dr. Glass received his Bachelors degree in Biophysics from the University of California, Berkeley and his M.D. and Ph.D. degrees from UCSD. Following internship and residency training in Internal Medicine at Harvard Medical School's Brigham and Women's Hospital, Dr. Glass returned to UCSD for clinical and research fellowships in Endocrinology and Metabolism. Dr. Glass' laboratory currently investigates roles of certain protein factors in regulating the development and function of an important group of immune system cells, work which he presented at the May 2010 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.

Inflammation and Chronic Disease

Dr. Glass introduced inflammation—and how it is normally regulated—in the context of its beneficial effects in fighting infection. The immune system defends the body against infection by identifying microbial invaders, and destroying them. White blood cells called macrophages migrate out of the circulatory system into tissues throughout the body to initiate this process by triggering inflammation at sites of infection or injury. Inflammation is often associated with heat, pain, redness, or swelling, and serves as a physiological siren to recruit other immune cells to the site of infection. The inflammatory response that follows is part of the way in which the body fights and recovers from the infection. When the wound heals, or the infection has been cleared, these symptoms disappear, as macrophages complete the process by deactivating the inflammatory response.

Inflammation is not always good for the body, however. In the last several years, researchers have discovered that some chronic, unhealthy conditions such as obesity also trigger a low-level inflammatory response in the absence of classic signs of inflammation, such as fever or pain. For example, macrophages accumulate in adipose (fat) tissue of obese individuals, triggering an inflammatory "defense" mechanism. Unlike the situation in a resolved infection, inflammation associated with obesity persists. This prolonged inflammation can lead to abnormal cell function and is thus thought to contribute to several common, chronic diseases, including heart disease and type 2 diabetes. A macrophage protein called Toll-like receptor 4 (TLR4), which recognizes an important class of bacteria and can initiate inflammation, also recognizes a variety of substances that are non-infectious, such as free fatty acids in the body, and may thus contribute to atherosclerosis, which is associated with heart disease; insulin resistance, which can lead to type 2 diabetes and is also a hallmark of this disease; and other chronic diseases, such as arthritis.

Dr. Glass discussed research from his laboratory that is unraveling at the molecular level how macrophages regulate inflammation and, in particular, attenuate the process when it is no longer needed, such as when an infection has resolved. His studies are also shedding light on the causes of many chronic diseases, and helping to identify potential avenues of intervention to treat or prevent them.

An early clue about the counter-regulation of the inflammatory process from the 1940s helped establish the theoretical underpinnings of the Glass laboratory's research. Researchers Edward Kendall

and Philip Hensch discovered that the steroid hormone cortisone could help relieve the inflammation and pain of rheumatoid arthritis. Unfortunately, long-term use of cortisone can result in significant undesirable side effects. Cortisone, it was later found, works by activating a type of protein called a "nuclear receptor."

The Nuclear Receptor Signaling Atlas (NURSA)

Nuclear receptors are proteins that help certain hormones—including cortisone, estrogen and testosterone, and many other substances, such as vitamin D—exert some of their effects. Typically, when one of these hormones (or other substances) enters a cell, it binds to its "receptor," and the hormone-receptor complex then interacts both with specific DNA sites and with other proteins to turn some genes in the cell's nucleus "on" and others "off." That is, the hormone-receptor complex triggers the cells to begin more actively utilizing genes that had been either dormant or only on at a relatively low level, or to stop utilizing a gene that was active prior to the signal. "Expression" level refers effectively to the extent that the cellular machinery is actively making the product or products of a particular gene.

With support from NIDDK, the National Heart, Lung, and Blood Institute, and the National Institute of Environmental Health Sciences, the Nuclear Receptor Signaling Atlas (NURSA) project is cataloging the diverse and extremely important physiological effects of nuclear receptor proteins, the hormones and other signals that stimulate them, and their many physiological effects, as well as their roles in disease.

NURSA research has helped document the tissues and cell types in which the 48 human nuclear receptors are found, and have shown that 28 of them are present in macrophages. Many undergo significant changes in their own expression levels in response to inflammatory stimuli. Each responds to its own set of chemical or hormonal signals to turn on and off different groups of genes. Dr. Glass' laboratory is studying the impact of macrophage nuclear receptors on inflammation.

Nuclear Receptors and Inflammation

Dr. Glass and his team found that stimulating macrophages with a molecule from pathogenic bacteria that is a powerful trigger of TLR4 boosted expression of over 500 different genes, many of them linked to the inflammatory response. The team then selectively stimulated three different nuclear hormone receptors, and explored the impact each one had on the array of genes that had been turned on by the bacterial trigger molecule. Each nuclear receptor reduced expression of hundreds of the inflammatory genes, but none turned off all of them. Some of the genes were turned off by just one of the receptors, some by two, and some by any one of the three. However, many remained unaffected by any of the three nuclear receptors examined in the experiment. Importantly, in addition to turning off various genes, each nuclear receptor also turns on a set of genes. Thus, it became clear that any set of stimuli is likely to yield a unique, complex, and finely tuned physiological response.

The potential of the nuclear receptors to tamp down an inflammatory response makes them particularly attractive as drug targets for treatment of pain, prevention of transplanted organ rejection, or prevention of atherosclerosis or type 2 diabetes. However, the complex cellular reaction from stimulating any individual receptor can make for undesirable side effects, as illustrated by the result of cortisone treatment for rheumatoid arthritis. Thus, researchers aim to understand how nuclear receptors do what they do, so as to harness them more selectively for therapeutic benefits while minimizing side effects.

An important example of a nuclear receptor that has been selectively targeted for this sort of strategy is PPAR-gamma, which is involved not only in control of inflammation by macrophages, but also in fat cell

development and, importantly, in regulating the levels of glucose in the body through its effects on fat, liver, and muscle cells. A class of diabetes pharmaceuticals known as thiazolidinediones, including rosiglitazone (sold as Avandia®) and pioglitazone (sold as Actos®), are PPAR-gamma stimulants that have been shown to be effective at helping people with type 2 diabetes control their blood glucose. Unfortunately, their use is also associated with increases in body fat, swelling associated with water retention in tissues (edema), and reduced bone formation. Rosiglitazone also has been linked, in some studies, to a higher risk of cardiovascular events, such as heart attack and stroke.

The Many Facets of the Nuclear Receptor PPAR-gamma in Health and Disease

In experiments in mice, Dr. Glass and his colleagues selectively eliminated PPAR-gamma in macrophages, leaving it intact in other cells, and found that the result was exaggerated inflammatory gene expression, and a loss of impact of the thiazolidinedione drugs on inflammation. This indicates that PPAR-gamma in macrophages has a much more profound effect on inflammation than PPAR-gamma in other cell types. Dr. Glass and his coworkers then looked at the impact of thiazolidinediones on glucose levels in two different groups of mice with type 2 diabetes: one group had PPAR-gamma in their macrophages, and the other did not. Both groups of mice had normally functioning PPAR-gamma in other tissues. The researchers found that the medications helped lower blood glucose in both groups of mice, but were more effective in the mice that had the nuclear receptor in their macrophages. This indicates that macrophage PPAR-gamma has a significant impact on glucose control—a surprise, because it was thought that liver, muscle, and fat cells are the sites where PPAR-gamma has its greatest impact on glucose levels.

They next examined a set of naturally occurring mutations in the PPAR-gamma gene that are known to affect human health. Most such mutations result in lipodystrophy, a disease in which fat cells are not normally distributed in the body. In PPAR-gamma-associated lipodystrophy, there is essentially no fat under skin in the extremities, but there is extra fat in the central parts of the body. Many of the mutations also cause type 2 diabetes and/or heart disease. In further analysis, Dr. Glass noted that all of the PPAR-gamma mutations that cause lipodystrophy also reduced PPAR-gamma's capacity to boost expression of other genes in the presence of receptor stimulants like rosiglitazone. One such mutation, however, reduced the capacity of PPAR-gamma to stimulate gene expression while leaving intact the ability of this nuclear receptor to reduce inflammation; notably, this mutation did not cause type 2 diabetes. This observation suggests that it may be possible to separate the functions of PPAR-gamma to reduce inflammation without stimulating other pathways through gene expression, and thus to treat type 2 diabetes and other inflammatory diseases, but avoid the side effects of thiazolidinedione treatment. A major goal of Dr. Glass' laboratory is to achieve exactly that.

Conclusion

Going forward, the Glass laboratory is also zeroing in on the specific cellular functions of PPAR-gamma and other nuclear receptors. New tools are allowing them to determine the many proteins these receptors are in physical contact with in the cell, and helping determine exactly what role they play in various specific tissues within a living animal. These avenues of inquiry will help complete the complex picture of what these important proteins do in health and disease.

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Nilia Olsen

Participating in TEDDY To Identify What Triggers Type 1 Diabetes in Children

Nilia Olsen

Four-year-old Nilia Olsen has no idea she's participating in a study that has determined she has an elevated risk for developing type 1 diabetes. She just knows that, every 3 months, she goes to the doctor's office to have her blood drawn, and, of all things, "she loves it," says her mom, Sonya.

"She likes the different colors on the tops of the vials," says Sonya, referring to the collection vials for blood samples. "Her favorite color is pink, so she likes to fill that one up first."

Nilia is one of over 8,000 children participating in The Environmental Determinants of Diabetes in the Young study, otherwise known as TEDDY. TEDDY is led by NIDDK and supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*.

The international study's long-term goal is to try to identify infectious agents, dietary factors, or other environmental agents, including psychosocial factors, that trigger type 1 diabetes in genetically susceptible individuals or protect against the disease.

But such details don't concern Nilia right now. She's a typical little girl who attends pre-school, likes to dress up—as well as dig for worms. "She's high energy," her mother laughs.

About the TEDDY Study

Researchers have discovered that children who develop type 1 diabetes have certain kinds of "high-risk" genes. Analyzing DNA from Nilia's blood shortly after she was born indicated that she was genetically at high risk to develop the disease. Researchers also know that some children with high-risk genes develop type 1 diabetes, while others don't. This has led them to think that something in the environment "triggers" or causes a child with high-risk genes to actually get type 1 diabetes. The purpose of TEDDY, therefore, is to try to identify the environmental triggers that cause children to get the disease. TEDDY has enrolled genetically susceptible newborns into the study from two populations: those with a sibling or parent with type 1 diabetes and those from the general population with no family history of the disease. Nilia falls into the general population group because she has no family history of type 1 diabetes.

Like Nilia, the other children in this study, all of whom were identified within 3 months of their birth as being at high genetic risk for developing type 1 diabetes, will be followed until age 15. During that time, information will be collected about their diets,

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illnesses, allergies, and other life experiences. Blood samples will be collected every 3 months and stool samples will be collected monthly for the first 4 years. After 4 years, these samples will be collected every 6 months until the children turn 15 years old. Parents are also asked to fill out questionnaires at regular intervals, and to record events, such as illnesses, in the child's "TEDDY Book."

From these numerous samples and other information collected about the children, researchers hope to identify a factor or factors that lead some genetically predisposed children to develop type 1 diabetes while others do not. This information is critically important for identifying strategies for disease prevention. For example, if a virus were found to trigger type 1 diabetes, a vaccine could possibly be developed. If a dietary factor were found to be causative, then changes to children's diets could be made.

It is only through the dedicated efforts of families, such as the Olsen family, that the TEDDY study could be conducted. Responsibilities such as making regular doctor's visits for blood draws, taking monthly stool samples for 4 years, and keeping detailed notebooks with information about their child's health is no small task. It is clear that TEDDY families are dedicated to the study and its goals. This commitment can reap major rewards if an environmental trigger is discovered, which could pave the way toward being able to prevent type 1 diabetes and help future generations of children.

One Family's Experience with the TEDDY Study

The day after Nilia was born, Sonya was asked if her daughter's blood could be sampled for a study to see if the child had an elevated risk for type 1 diabetes. Although there is no history of type 1 diabetes in the family, Sonya was aware that nearly all of the women on her father's side have type 2 diabetes (formerly called adult-onset diabetes). Sonya immediately agreed and enrolled Nilia into TEDDY when researchers found that Nilia carried high-risk genes for type 1 diabetes.

"I don't have diabetes, and neither does my husband, Thomas," says Sonya. Thomas is in the military and was deployed to Iraq on his second tour of duty in October 2009. Beyond her daughter's increased genetic risk for type 1 diabetes, Sonya says that an additional motivation for her to enroll Nilia into TEDDY was "knowing that type 2 diabetes runs on my father's side of the family." Both forms of diabetes can lead to serious health complications.

"I would strongly encourage other families to participate in clinical trials like TEDDY," says Sonya. "Knowing that our daughter is in a trial like TEDDY gives us a great deal of peace of mind."

At the time this profile was written, it was 4 years since Nilia's blood was originally sampled, and although it was discovered that she has an elevated risk for developing type 1 diabetes, fortunately she remains diabetes free. Sonya says that knowing Nilia has an elevated risk "was something that as a family we knew we would take in stride." Not only have they taken it in stride, they have remained dedicated to contributing to an important research study that can lead to new ways to prevent type 1 diabetes. According to TEDDY staff, Sonya has always gone the extra mile to do everything she could for TEDDY research, including participating in optional fun events designed to build community among local TEDDY families. However, the Olsen family lived in Augusta, Georgia, when Nilia first entered the study. Since then, the family has been transferred by the military to Alabama. "The study staff has been very flexible," says Sonya. "We do everything through the mail. Every time Nilia's blood and stool samples get tested, they mail us the results. It's great. The continuity provides us with a sense of peace."

As with all other study participants, the Olsen family was provided a calendar, "and we record whenever

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Nilia goes to the doctor," says Sonya. "We write down when she's sick and what kind of medication she may be taking. We record if she goes to the hospital." But so far, according to Sonya, Nilia has not had any hospitalizations or health issues related to diabetes. As for filling out the calendar and other paperwork related to the study, "It's not difficult, at all," says Sonya. "It just takes a few minutes to record."

Nilia may be a big reason the family is able to cope so well. At age 4, she is proving to be a real trooper when it comes to participating in the study. "Whenever she goes to the bathroom, she asks if she needs to poop in the cup," Sonya says, with a slight laugh. "And she's really good at having her blood drawn. She never cries about it and never has." In fact, Nilia is so good about having her blood drawn that she is featured in a video about blood draws for TEDDY that has been distributed to other TEDDY sites.

"I would strongly encourage other families to participate in clinical trials like TEDDY," says Sonya. "Knowing that our daughter is in a trial like TEDDY gives us a great deal of peace of mind."

Nilia's father, Thomas, calls home once a week from Iraq and asks how Nilia is doing. "It pleases me to be able to tell him she's doing well."

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Joan Pasquesi

HAPO Study Brings Results—and Comfort

Joan Pasquesi and her three children

When Joan Pasquesi, a nurse at Northwestern Memorial Hospital, in Illinois, learned that a study related to gestational diabetes was going to be conducted at her research center, she jumped at the opportunity to participate—and for good reason.

"I've never had any health issues related to diabetes," says Joan, "but my father passed away at age 63 from complications of type 2 diabetes."

At the time the study started, Joan was pregnant with her first child. Knowing that diabetes has a strong genetic link, and that many of her father's cousins and aunts also had type 2 diabetes, Joan saw the study as a step she could take to help protect her health and that of her future children. She now has two sons and a daughter ranging in age from 4 to 10 years, all of whom are diabetes free. Still, Joan remains watchful, aware that her family history means that she and her children are likely at greater risk of developing diabetes. Says Joan, "Knowing what my father went through forces me to be more vigilant."

The study that Joan enrolled in was a noninvasive, observational study called the Hyperglycemia and Adverse Pregnancy Outcomes, or HAPO, study. While it is known that overt diabetes places women and their babies at higher risk for complications during pregnancy and delivery, there has been longstanding debate as to whether such risks apply to gestational diabetes. Spearheaded by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), with co-support from NIDDK, the purpose of HAPO was to clarify whether even mildly elevated maternal blood glucose (sugar) levels—*i.e.*, lower than the levels currently used to diagnose diabetes—increase the risk of adverse outcomes for pregnant women and their babies.

Says Joan, "Knowing what my father went through forces me to be more vigilant."

About Hyperglycemia and Gestational Diabetes

Gestational diabetes mellitus (GDM) is a form of diabetes that occurs during pregnancy. Like other forms of the disease, it is characterized by high blood glucose levels and can have serious consequences. Left untreated or uncontrolled, GDM can result in babies being born very large—9 pounds and over—and with extra fat, which can make delivery difficult and riskier for both mother and child.

While the exact cause of GDM is not known, it is thought that hormonal changes that occur during pregnancy are a major contributor. These changes impair the action of insulin in body tissues, causing a pregnant woman's blood glucose levels to rise—a condition known as hyperglycemia. In approximately 7 percent of pregnancies, levels are high enough, by current criteria, to indicate the presence of diabetes. While any pregnant woman may be at risk for GDM,

strong risk factors include obesity and a family history of type 2 diabetes.

For most women, GDM resolves after the baby is born, but leaves them at greater risk for the disease during subsequent pregnancies. GDM also increases the offspring's risk for obesity, and leaves both mother and child at increased risk for diabetes for the rest of their lives—risks that, given her father's experience with type 2 diabetes, did not escape Joan.

The Need To Stay Vigilant

"My father was one of those guys who never went to see a doctor," says Joan. "He felt he had no need. At age 44 he was feeling fine. He had never been admitted into a hospital nor missed a day of work in his life," she adds. "He was one of those guys whose attitude was 'if it ain't broke…'."

Despite her father's reluctance, Joan's mother convinced him to have a routine check-up.

"I was only 9 years old at the time, but I can remember it as if it happened yesterday," says Joan. "Shortly after his first general check-up in years, we received an urgent call from my father's physician, who told him to get into the hospital immediately." The doctor told Joan's family that her father's glucose levels were extremely high and "out of control," she continues. "He was at immediate risk for suffering a stroke or heart attack. We were all scared to death, including my father."

As it turned out, Joan's father lived to age 63. But, before he died, both his legs had to be amputated, he suffered multiple heart attacks and strokes, and he was on kidney dialysis—all of which are recognized as serious complications of type 2 diabetes. Joan wasn't about to risk a similar fate for herself or her children.

The HAPO Study

Joan's enrollment in the HAPO study placed her among a very diverse group of more than 25,000 other women who also were having their blood glucose levels examined during pregnancy in the study's 15 centers located in nine countries around the world. In addition, Joan was selected to take part in a training video to help ensure that the study would be carried out uniformly at all 15 centers.

According to Boyd E. Metzger, M.D., at the Northwestern University Feinberg School of Medicine, who led the study, training was critical to HAPO. "From drawing blood, to sending it to the lab, shipping it, and entering the data forms, all had to be consistent and conform to protocol," he says.

As for the study itself, Joan says, for the added comfort and security it provided her during her pregnancy, it required minimal time or effort. "The study was well run by knowledgeable coordinators and nurses," she adds. "To have these people as additional resources, and to know that my personal physician coordinated with them during my pregnancy was reassuring."

At about 28 weeks into their pregnancies, HAPO study participants were given an oral glucose tolerance test. This test is used to determine if a person has higher than normal levels of glucose in their blood 1 to 3 hours after drinking a standardized sugary drink. This test not only provided information about blood glucose levels below the threshold for diabetes, but also helped HAPO researchers to find women whose blood glucose levels were already high enough to meet a predefined threshold for treatment. In Joan's case, they were not. Had they been, she, like any participant in the study who met or exceeded the threshold, would have been made aware of the results so that her physician could begin treatment. Otherwise, it was a "blinded" study, meaning that unless the testing revealed overt diabetes requiring treatment, or a different test conducted later in pregnancy revealed an emerging problem with hyperglycemia, the HAPO participants, as well as the HAPO study staff (except laboratory

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personnel), remained unaware of their glucose levels during the study.

To see whether elevated maternal blood glucose levels below levels diagnostic for diabetes are associated with adverse pregnancy outcomes, the HAPO researchers also collected information about each participant's pregnancy and delivery. For example, they looked at whether the mother needed a cesarean section, or whether a baby experienced certain types of injury during vaginal delivery. Also, at the time of their birth, blood was taken from the baby's umbilical cord and tested for elevated insulin levels. A heel-stick blood glucose test was done approximately 2 hours after birth to detect hypoglycemia. High fetal insulin levels and a low newborn blood sugar level are two potential effects of maternal hyperglycemia on the baby. A study coordinator came within 24 hours of the birth to measure the baby's weight and skin fold thickness, an estimate of fat tissue. "A month later, they followed up to ask how my baby and I were doing," says Joan. "It was all very comforting and well coordinated." Ultimately, blood glucose data and pregnancy and birth outcomes from over 23,300 women were included in the HAPO study.

Results of the Study

The HAPO study demonstrated that even modest elevation of maternal blood glucose below the level that is diagnostic of diabetes is associated with adverse birth and pregnancy outcomes—meaning that the optimal levels of blood glucose during pregnancy are lower than previously appreciated.

The results of the HAPO study were "very convincing," says Dr. Metzger. The higher a pregnant woman's blood glucose levels, the more likely she is to deliver a high birth-weight baby, and the more likely the baby is to have signs of hyperinsulinemia, a condition in which there are excessive levels of circulating insulin in the blood.

HAPO demonstrated that even modest elevation of maternal blood glucose levels is associated with adverse birth and pregnancy outcomes—meaning that the optimal levels of blood glucose during pregnancy are lower than previously appreciated.

The study results also suggest that elevated blood glucose is associated with a greater risk of problems in the mother, such as preeclampsia, a potentially serious condition that only occurs during pregnancy in which high blood pressure and protein in the urine develop after the 20th week (late 2nd or 3rd trimester) of pregnancy. Usually the high blood pressure, protein in the urine, and other effects of preeclampsia go away completely within 6 weeks after delivery. However, sometimes the high blood pressure will get worse in the first several days after delivery.

As a result of the HAPO study, an international panel of researchers and physicians was assembled to examine its findings and the findings of related studies. The panel's recommendations for developing a new strategy for diagnosing GDM, which includes lowering the diagnostic threshold for GDM based on hyperglycemia testing, have been adopted by the American Diabetes Association.

"Given all I know about the deadly complications of diabetes as a result of my father's experience, this study was a no-brainer for me. I'd participate again, if I could."

The HAPO study results are also providing a framework for research questions focused on improving health outcomes for pregnant women and their offspring. Researchers believe, for example, that an observed increase in the rate of

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GDM globally is driven in large part by increased rates of overweight and obesity in women of childbearing age. These factors may also contribute to elevated glucose levels like those seen in women in the HAPO study. One approach to minimizing risk for all these women and their babies would be to monitor and minimize excessive weight gain during pregnancy—questions that may be pursued through research. Based on the HAPO results, researchers can also now explore questions such as the level of risk of future type 2 diabetes associated with GDM diagnosed by the new criteria or other long-term health risks in the mother and child.

As for Joan, "Given all I know about the deadly complications of diabetes as a result of my father's experience, this study was a no-brainer for me," she says. "I'd participate again, if I could."