

Up to half of people with diabetes suffer damage to nerves throughout the body, damage that can lead to decreased sensation, difficulty moving, and pain and tingling in arms, legs, hands, and feet—a condition called diabetic sensorimotor polyneuropathy, or DSPN. Neuropathy is a major risk factor for amputation. Because the earliest signs of nerve damage occur in small nerves, tools to assess small nerve damage are critical for detecting and developing new therapies for DSPN. Currently, the most reliable tool for assessing small nerve damage is an invasive skin biopsy. A noninvasive technique to measure small nerve damage has the potential to improve the ability to detect and monitor DSPN early and to speed research to develop new approaches to prevent and treat it. Research described in this chapter indicates that a noninvasive laser-based imaging technique called corneal confocal microscopy (CCM), which allows users to image small nerves in the front of a person's eye, could be a new tool for detecting DSPN. The three panels above show CCM images of nerve fibers (white) in the eyes of three study participants. Compared to a person without diabetes (left) and a person with type 1 diabetes but no DSPN (middle), a person with type 1 diabetes and DSPN (right) has lower nerve fiber density, as illustrated by fewer arrows pointing to main (red arrow) and branch (yellow arrow) nerve fibers. As described in the chapter, both CCM and the invasive skin biopsy technique were similarly effective for diagnosing DSPN. These new findings suggest that the noninvasive nature of CCM, as well as its potential for automation, could make it preferable to skin biopsy for detecting DSPN.

Images courtesy of Dr. Xin Chen, University of Manchester and Dr. Rayaz A. Malik, University of Manchester and Weill Cornell Medicine Qatar. From: Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, Alam U, Marshall A, Fadavi H, Ferdousi M, Azmi S, Tavakoli M, Efron N, Jeziorska M, Malik RA. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. American Diabetes Association [Diabetes Care](#), American Diabetes Association, 2015. Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association.

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 29.1 million people in the United States—or 9.3 percent of the total population—and is the seventh leading cause of death.¹ Compared with people of similar age without the disease, overall rates of death are about 1.5 times higher in people with diabetes, and rates of death from cardiovascular disease are 1.7 times higher.¹ Although rates of diabetes-related complications have declined substantially in the past two decades, disease burden remains significant as the number of people with diabetes continues to increase.² Diabetes can affect many parts of the body and is associated with serious complications, such as heart disease and stroke, blindness, kidney failure, and lower-limb amputation. In addition to these human costs, the estimated total financial cost for diabetes in the United States in 2012—including costs of medical care, disability, and premature death—was \$245 billion.³ Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.

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. In addition, a significant proportion of pregnant women each year are diagnosed with gestational diabetes, a form of diabetes that is similar to type 2 diabetes but unique to pregnancy. Untreated, any form of diabetes during pregnancy increases the risk of serious complications for the mother and baby before, during, and after delivery.

Type 1 diabetes, formerly known as juvenile diabetes, affects approximately 5 percent of diagnosed

¹ Centers for Disease Control and Prevention. *National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014*. Atlanta, GA: U.S. Department of Health and Human Services, 2014.

² Gregg EW, et al. *N Engl J Med* 370: 1514-1523, 2014.

³ American Diabetes Association. *Diabetes Care* 36: 1033-1046, 2013.

diabetes cases in adults, and the majority of diagnosed cases in children and youth.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 β (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, people with type 1 diabetes require lifelong insulin administration—in the form of multiple daily injections or *via* an insulin pump—to regulate their blood glucose levels. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that keeping blood glucose levels as near to normal as safely possible reduced the risk of eye, kidney, nerve, and heart complications associated with type 1 diabetes. However, despite vigilance in disease management, with current technologies to test blood glucose levels and administer insulin, it is still not possible for people with type 1 diabetes to control blood glucose levels as well as functional β cells do. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery. This includes continued development and testing of “artificial pancreas” technologies in real-world settings, as well as working to develop β cell replacement therapies, such as islet transplantation, to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.¹ The risk for developing type 2 diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity.

Type 2 diabetes occurs at higher rates among racial and ethnic minority populations in the United States, including African Americans, Hispanic and Latino Americans, American Indians, and Native Hawaiians and Pacific Islanders.¹ Gestational diabetes is also a risk factor: shortly after pregnancy, 5 to 10 percent of women with gestational diabetes continue to have high blood glucose levels and are diagnosed with diabetes, usually type 2.¹

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the pancreatic β cells lose their ability to secrete enough insulin to restore balance, and the timing of insulin secretion becomes abnormal, causing blood glucose levels to rise. 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 86 million U.S. adults who have a condition called “prediabetes,” in which blood glucose levels are higher than normal but not as high as in diabetes.¹ This population is at elevated risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with prediabetes 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. To a more limited degree, the safe and well-tolerated drug metformin can also help prevent or delay type 2 diabetes. Moreover, follow-up research has shown that the benefits of reduced diabetes risk from weight loss or metformin 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 youth from racial and ethnic minority populations in the United States. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. For example, the NIDDK-supported Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial showed that the disease may be more aggressive and difficult to treat in youth compared to adults. This is worrisome because the onset and severity of disease complications correlate with both the duration of diabetes and control of blood glucose levels; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. In addition, increasing rates of type 2 diabetes in girls may lead to more women who enter pregnancy with diabetes, and 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 type 2 diabetes in offspring. Thus, the rising rates of diabetes and prediabetes in young women could lead to a cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the United States.

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 NIDDK'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 NIDDK is vigorously pursuing studies of prevention and treatment approaches for these diseases.

DIABETES PREVALENCE

First National Data on Diabetes Rates in Asian Americans: New research has found that more than half of Asian Americans with diabetes are undiagnosed, and that the prevalence of diabetes for all American adults increased between 1988 and 2012. In the new study, researchers analyzed data from 26,415 adults in the National Health and Nutrition Examination Survey (NHANES), which is a study to monitor nutritional and health status in the U.S. population. From this ongoing survey, they were able to generate national data on diabetes rates from 1988 to 2012. Understanding the prevalence (proportion of the population with the disease) of diabetes in the United States, how it changes over time, and which populations are disproportionately affected by the disease, could inform future research, public health, and educational awareness efforts to combat it.

The analyses showed that, between 1988 and 2012, diabetes prevalence for U.S. adults increased from nearly 10 percent to over 12 percent when measured by the tests commonly used to diagnose diabetes in clinical practice; more sensitive research tests yielded somewhat higher rates. Diabetes prevalence in U.S. adults also went up in both sexes and every age, level of education, income, and racial/ethnic subgroup. On positive notes, the proportion of people with diabetes that was undiagnosed decreased by 23 percent during the same time period, and diabetes prevalence has remained largely unchanged in more recent years (between 2007 and 2012).

For the 2011-2012 survey, NHANES surveyed a disproportionately large number of Asian Americans, which allowed researchers to quantify diabetes prevalence in this population for the first

time. Using the more sensitive research tests to define diabetes rates, they found that nearly 21 percent of Asian Americans had diabetes, with 51 percent of those individuals undiagnosed—the highest proportion of undiagnosed diabetes among all ethnic and racial subgroups studied. However, one difference between Asian Americans and the other groups studied is that Asian Americans often develop type 2 diabetes at a lower body mass index (BMI, a measure of weight relative to height). The NHANES data showed that the average BMI for all Asian Americans surveyed was just under 25; for the U.S. population overall, the average BMI was just below 29. (A BMI of 25 to under 30 is considered overweight, and a BMI of 30 or greater is considered obese.) These findings underscore the American Diabetes Association’s recommendation that Asian Americans get tested for type 2 diabetes at a BMI of 23 or higher, which is lower than the BMI threshold of 25 or higher that is recommended for the general population.

The study also provided data on other ethnic and racial subgroups in the United States. Again using the more sensitive research test to define diabetes rates, the scientists found that Hispanic Americans had the highest prevalence of diabetes at nearly 23 percent, with 49 percent of those individuals undiagnosed. Nearly 22 percent of non-Hispanic black adults had diabetes; however, they had a lower proportion of diabetes that was undiagnosed than the Asian or Hispanic subgroups, at about 37 percent. Non-Hispanic whites had the lowest prevalence of diabetes at 11 percent, and the lowest proportion of diabetes that was undiagnosed, at just over 32 percent.

These findings give important information about how diabetes affects the U.S. population and provide the first national data on total diabetes (diagnosed and undiagnosed) prevalence in Asian Americans.

The high prevalence of undiagnosed diabetes in Asian Americans suggests the need for increased awareness about type 2 diabetes screening at a lower BMI threshold in this population.

Menke A, Casagrande S, Geiss L, and Cowie CC. Prevalence of and trends in diabetes among adults in the United States, 1988-2012. JAMA 314: 1021-1029, 2015.

TYPE 1 DIABETES—HEALTH BENEFITS OF GOOD GLUCOSE MANAGEMENT

Early and Intensive Glucose Control Can Result in Better Eye Health and Longer Life for Those

with Type 1 Diabetes: People with type 1 diabetes who intensively control their blood glucose (sugar) early in their disease are likely to live longer and require fewer eye surgeries than those who do not. These findings are the latest results of the Diabetes Control and Complications Trial (DCCT) and its follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study. In type 1 diabetes, the body does not make insulin, and people with the disease need to take daily insulin to live. Beginning in 1983, the DCCT enrolled 1,441 males and females between ages 13 and 39 with type 1 diabetes. The goal of the trial was to determine whether intensive blood glucose control would result in fewer complications later in life. In the DCCT, half of the participants were assigned at random to intensive blood glucose control designed to keep blood glucose levels as close to normal as safely possible, and half were assigned to what was conventional treatment at the time. The DCCT ended in 1993 when the intensive control group was found to have substantially less eye, nerve, and kidney disease. Because of this result, intensive blood glucose control is now the standard of care for people with type 1 diabetes, though achieving such intensive control can be difficult. After the DCCT

ended, all participants were taught intensive blood glucose control and the researchers have continued monitoring their health during the ongoing EDIC study, which showed that blood glucose control has since been similar in both groups.

To further study the lasting effects of the different treatments tested by the DCCT, the researchers examined differences in overall lifespan between the intensive and conventional control groups. They found that after an average of 27 years of follow-up, the former intensive control group had a 33 percent reduction in deaths from all causes (43 versus 64) compared to the former conventional treatment group. The most common causes of death were cardiovascular disease (22 percent), cancer (20 percent), dangerously high or low blood glucose (18 percent), and accidents/suicide (17 percent). Additionally, fewer people in the former intensive treatment group than the former conventional treatment group died from diabetic kidney disease (one versus six). Higher average blood glucose levels and increased protein in the urine—a marker of diabetic kidney disease—were the major risk factors for death. A related study involving the DCCT/EDIC cohort showed that, after an average of 23 years of follow-up, participating in the intensive glucose control treatment group during the DCCT was associated with a 48 percent reduction in the risk of diabetes-related eye surgery, such as surgery to treat retinopathy, cataracts, or glaucoma. This reduction in eye surgeries led to a 32 percent reduction in the costs of surgery for those who had been in the DCCT's intensive control group, demonstrating how prevention of diabetes complications can have significant cost implications over the long term. Analysis of the rates of eye surgeries in conjunction with markers for blood glucose control indicated that improved glucose control accounted for virtually all the benefit seen from the intensive treatment.

These results from DCCT/EDIC further emphasize the importance of good glucose control in maintaining health and underscore the need for new tools and technologies to help people with type 1 diabetes achieve recommended blood glucose levels. The findings also add to the growing evidence that even a finite window of intensive glucose control early in the course of type 1 diabetes can have lasting benefits, resulting in reduced rates of diabetes complications and contributing to longer, healthier lives.

Writing Group for the DCCT/EDIC Research Group: Orchard TJ, Nathan DM,...Lachin JM. Association between 7 years of intensive treatment of type 1 diabetes and long-term mortality. [JAMA](#) 313: 45-53, 2015.

Writing Group for the DCCT/EDIC Research Group: Aiello LP, Sun W,...Nathan DM. Intensive diabetes therapy and ocular surgery in type 1 diabetes. [N Engl J Med](#) 372: 1722-1733, 2015.

COMBATING HYPOGLYCEMIA ASSOCIATED WITH INSULIN THERAPY

Discovery of Brain Pathway That Responds to Low Blood Glucose Levels: Researchers have discovered a novel role for the hormone leptin in combating low blood glucose (sugar) and found that leptin acts through a previously unknown brain pathway to exert this effect, with potential implications for diabetes management. People with type 1 diabetes require insulin for survival; research has shown that intensive insulin therapy to control blood glucose levels is critical for improving their long-term health. However, too much insulin increases the risk of hypoglycemia (low blood glucose), which could result in life-threatening consequences. When glucose levels fall too low, the body reacts with a counter-regulatory response (CRR) to raise them; the brain is known to play an

important role in glucose sensing and in regulating the CRR. However, in people with type 1 diabetes, the CRR is impaired and worsens with each episode of low blood glucose. Thus, it is important to identify ways to preserve a robust CRR to protect people from adverse consequences of hypoglycemia so that they can achieve intensive blood glucose control.

Hypoglycemia can also occur in a fasted or starved state. Researchers hypothesized that, under such conditions, the body may respond by mounting a more robust CRR to overcome the nutrient deprivation, and that leptin may play a role. Leptin is a hormone secreted by fat cells; low leptin levels signal to the body that energy stores are low and promote hunger. Researchers speculated that low leptin levels may also enhance the CRR as a means to protect against hypoglycemia.

To test this hypothesis, the scientists conducted a series of experiments in mice. Previous research showed that leptin targets nerve cells (neurons) in several regions of the brain by binding to a cell surface protein called the leptin receptor. In a new study, researchers focused on a brain region, called the parabrachial nucleus (PBN), where they identified many neurons with the leptin receptor. They found that PBN neurons targeted a different brain region known to be involved in the CRR; that low glucose levels activated PBN neurons; and that leptin decreased their activity. These observations suggest that when glucose and leptin levels are low, PBN neurons are activated, which may enhance the CRR to protect against hypoglycemia. To examine this further, the scientists genetically engineered mice to lack the leptin receptor in a subset of PBN neurons (so that leptin cannot bind to the cells) and found evidence of an enhanced CRR when insulin was used to induce hypoglycemia. Because leptin has other functions, the researchers further examined the mice. They saw no effect on the

animals' body weights or on normal regulation of glucose levels in the absence of insulin-induced hypoglycemia. Other experiments showed that a hormone called cholecystokinin (CCK), which is involved in regulating food intake, is also expressed in PBN neurons with the leptin receptor; CCK was found to play an important role in this newly identified pathway. These findings suggest that low levels of leptin enhance the CRR through PBN neurons that express both CCK and the leptin receptor, and this effect is separate from leptin's well-known roles in regulating appetite and calorie burning (energy balance).

This research not only led to the discovery of a previously unknown brain pathway that plays a key role in regulating blood glucose levels, but also identified a novel role for leptin. Because PBN neurons were not involved in regulating energy balance or glucose levels under normal glucose conditions, the research suggests that this new pathway provides a potential specific therapeutic target to protect against hypoglycemia associated with insulin therapy. Further research could help determine the specific cells targeted by the PBN neurons that are involved in the CRR and if the findings are applicable in humans.

Flak JN, Patterson CM, Garfield AS,...Myers MG Jr. Leptin-inhibited PBN neurons enhance responses to hypoglycemia in negative energy balance. Nat Neurosci 17: 1744-1750, 2014.

REPLACING BETA CELLS

Molecule Shows Promise in Promoting Beta Cell Replication: Researchers have found that a small molecule called harmine may promote β (beta) cell replication, potentially creating new approaches for expanding β cell mass. In both type 1 and type 2 diabetes, critical insulin-producing β cells

are lost. Because β cell proliferation is greatly diminished after infancy, older children and adults cannot replace lost β cells through regeneration. Research to identify strategies for expanding the number of β cells, throughout the lifespan, is therefore important to the treatment of both types of diabetes. Although an attractive strategy, inducing human adult β cells to regenerate has proven to be a difficult goal. One strategy involves stimulating β cell replication—inducing existing mature β cells to divide and produce an exact copy of themselves.

To identify new agents that promote β cell replication, researchers in this study screened over 100,000 small molecules with a high-throughput assay measuring cell growth signals that drive the replication of human β cells. They found one promising molecule, called harmine, that represents a newly discovered class of molecules. Harmine and other closely related molecules were found to increase human β cell replication both in β cells cultured in the laboratory and in intact animals. Importantly, researchers were able to probe the mechanism of harmine's action: first, they identified a specific target of its action as DYRK1A (Dual-specificity tyrosine-regulated kinase-1a); and second, they determined that a group of proteins that regulate gene activity (transcription factors) that belong to the NFAT (nuclear factors of activated T cells) family serve as mediators of human β cell proliferation and differentiation (the process by which cells become specialized).

To explore whether harmine induced β cell replication in live animals, the scientists tested its effects in three different mouse models. In the first, part of the pancreas of male mice was removed to prompt β cell growth. When the mice were treated with harmine, the scientists observed a rapid and robust replication of β cells and an increase in β cell mass. In the second model, mice were transplanted with both

male and female human β cells. The transplanted human β cells increased their replication in response to harmine treatment. Finally, harmine treatment improved glucose (sugar) control in non-obese diabetic mice transplanted with human β cells. Based on these findings, researchers have demonstrated harmine can induce β cell replication of both rodent and human β cells, leading to increased β cell mass and improvements in blood glucose levels.

This study provides promising evidence that harmine may promote human β cell replication, opening potential new therapeutic approaches for expanding β cell mass. There are several challenges before harmine, or an optimized version of it, could be ready for market. Further research will be needed to determine if the results in rodents hold true in humans. Additionally, because the mechanism through which harmine induces cell replication is not unique to β cells, and because harmine is known to have effects elsewhere in the body, harmine delivery will need to be tailored specifically to β cells to avoid off-target effects. Additional studies are also needed to determine the proper dose of harmine and duration of treatment because too much β cell replication could be harmful.

*Wang P, Alvarez-Perez JC, Felsenfeld DP, ... Stewart AF. A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. *Nat Med* 21: 383-388, 2015.*

ADVANCING TYPE 2 DIABETES TREATMENTS

A Clearer Picture of the Molecular Underpinnings of Insulin Resistance and Type 2 Diabetes:

Recent experiments in mice have shed greater light on the regulation of insulin resistance and type 2 diabetes, suggesting a possible new approach to medical treatment for these

conditions. The thiazolidinediones, or glitazones, are insulin-sensitizing medications that act by targeting PPAR γ , a protein known to be one of the key molecular arbiters of insulin resistance and type 2 diabetes. While glitazones are effective at improving glycemic control in people with type 2 diabetes, they have also been associated with a variety of side effects. In previous studies, researchers showed that one effect of glitazone treatment is to block the addition of a phosphate group to a particular site on PPAR γ (a process called phosphorylation). Disruption of this phosphorylation event experimentally promotes the expression of genes that increase insulin sensitivity, leading to improved glycemic control, but without the side effects often seen with glitazones.

In a new study, the researchers sought to better understand the regulation of PPAR γ by creating mice whose adipose (fat) tissue lacked the protein they thought to be responsible for the key PPAR γ phosphorylation. Surprisingly, however, this caused an increase (rather than the expected decrease) in phosphorylation of PPAR γ and the mice became more (rather than less) susceptible to the diabetes-inducing effects of a high-fat diet. Through further experimentation, they were able to identify the enzyme responsible for the increased phosphorylation, a protein called ERK. Working with male mice that had obesity and diabetes either as a result of diet or due to a genetic mutation, the researchers tested two different compounds known to block activation of ERK. Both were well-tolerated by the mice and proved effective at treating their diabetes without affecting their body weight, suggesting one or both might be useful therapeutically for treatment of type 2 diabetes in people. One of these compounds is approved by the U.S. Food and Drug Administration for the treatment of melanoma, a type of skin cancer. Further testing will be needed, however, to determine whether either compound is safe and effective for long-term use in the treatment of people with diabetes.

Banks AS, McAllister FE, Camporez JP,...Spiegelman BM. An ERK/Cdk5 axis controls the diabetogenic actions of PPAR γ . Nature 517: 391-395, 2015.

Genetic Differences Throughout the Genome Affect the Function of a Key Metabolic Regulator:

New research has led to the discovery of an important way that genomic variation can affect metabolic health and response to one class of medication for type 2 diabetes; these findings could lead to development of personalized treatments for the disease. Our genes contain the encoded recipes for making thousands of different proteins. When genetic differences alter these recipes in ways that inactivate or change the activities of the proteins they encode, there can be significant consequences for human health. But a surprisingly large portion of the genome is made up of stretches of DNA that do not code for proteins, and it has been something of a mystery as to why many genetic differences that are known to influence risk for various diseases have been found in these non-coding regions. One part of the explanation likely stems from the fact that proteins are not produced at the same rate throughout the body: their respective genes are “expressed” (activated so that the protein they encode can be produced) to differing extents in different tissues, at various stages of development and disease, and in response to nutritional and environmental cues. This pattern of gene utilization is enforced by expression-regulating proteins that bind to DNA sequences typically located near to (but not usually within) the genes they regulate. Thus, changes in non-coding DNA could affect health by altering the binding sites of these regulatory proteins, thereby influencing the expression of nearby genes.

In the new study, researchers investigated binding sites (in both mice and humans) for one such regulatory protein, called PPAR γ , which plays a key role in controlling metabolic gene expression. They

began by comparing male mice from two genetically different strains, one of which was more susceptible to type 2 diabetes and other metabolic diseases than the other. PPAR γ can bind approximately 35,000 sites in the mouse genome, and the investigators found that in male mice genetic differences between the two strains affected PPAR γ 's ability to bind about 2,000 of these sites. In some cases, variations in the DNA sequence within PPAR γ binding sites (or within the binding sites of other regulatory proteins PPAR γ sometimes works with) had large effects on PPAR γ binding. The researchers were able to document that, in general, stronger PPAR γ binding correlated with stronger expression of nearby genes, indicating that sequence variation at these sites had a significant impact on gene expression in the two strains of mice. These differences in gene expression were accentuated when the mice were treated with rosiglitazone, a medication for type 2 diabetes that works by activating PPAR γ . What makes this particular finding so significant is that the response to rosiglitazone is quite variable: it dramatically lowers blood glucose (sugar) in most (but not all) people with type 2 diabetes, and the drug is linked to significant side effects in some who take it. These findings suggest that the positive and negative variations in rosiglitazone response may be linked to differences in PPAR γ binding sites near certain key genes.

To test whether people have significant, physiologically relevant differences in the binding sites of PPAR γ and the proteins it works with, the investigators compared samples from five obese female volunteers (one Hispanic, one African American, and three White). As in mice, numerous differences were found that affect how well PPAR γ binds various regulatory sites. The researchers looked at expression of genes near these sites using data from a previous study of samples from a group of 1,381 Finnish men with diabetes or at risk for the disease. They found that stronger

binding of PPAR γ or its partners correlated well with higher expression of nearby genes, suggesting that natural differences in PPAR γ binding sites correlate with markedly different gene expression patterns in people as well as mice. In fact, the analysis uncovered a previously undescribed PPAR γ binding site difference that appears to have a significant impact on levels of good cholesterol and other metabolic factors and thus, presumably, on human health. Further analyses of variation in human PPAR γ binding sites may lead to identification of genetic variations associated with beneficial and/or harmful responses to rosiglitazone, and may one day facilitate personalized treatment for type 2 diabetes by identifying those most likely to benefit from and least likely to be harmed by the drug. Indeed, these findings chart a course toward better understanding the effects of any drug that acts on proteins that regulate gene expression.

Soccio RE, Chen ER, Rajapurkar SR,...Lazar MA. Genetic variation determines PPAR γ function and anti-diabetic drug response in vivo. Cell 162: 33-44, 2015.

Discovery of Naturally Occurring Fats That May Alleviate Diabetes and Inflammation: Working with a mouse model system, investigators have identified a group of fatty acids (a subgroup of lipids, or fat molecules) that appears to improve blood glucose (sugar) control and reduce inflammation. Previously, they noticed that mice from a particular genetically engineered strain were obese, with high levels of fatty acids, but—unlike many other obese mice—these had very good blood glucose control. The investigators wondered whether one or more of the elevated fatty acids might be playing a role in maintaining glucose control in these mice, so they compared lipids recovered from the adipose (fat) tissue from the engineered strain to lipids from normal control mice. This analysis led to the discovery of a family of fat molecules called PAHSAs

(palmitic acid-hydroxy stearic acids), which were present in both groups of animals but found at much lower levels in the control mice. In a test of normal mice with diet-induced obesity and type 2 diabetes, they found several of the PAHSAs were significantly reduced in some tissues compared to levels seen in normal mice on a healthier diet. The researchers also found similar relationships between PAHSA levels and insulin resistance in a small group of human volunteers, suggesting that low PAHSA levels may be associated with increased risk for type 2 diabetes.

To test whether PAHSAs may have therapeutic potential, the researchers orally administered two forms of these fatty acids (designated 5-PAHSA and 9-PAHSA) to mice with diet-induced type 2 diabetes. They found that each of the compounds lowered fasting glucose levels in the mice, and greatly improved their ability to manage glucose levels after feeding. The results suggest that these PAHSAs improve blood glucose control in at least two ways: by increasing the animals' insulin sensitivity, and—when they are fed—by increasing their insulin-production response. When investigators examined insulin-producing β (beta) cells from non-diabetic human donors (one man and one woman), they found that exposure to 5-PAHSA resulted in a modest increase in glucose-stimulated insulin production. They observed a more pronounced effect in mouse intestinal cells: both 5- and 9-PAHSA stimulated these cells to produce the hormone GLP-1, which itself can stimulate β cells to produce more insulin in response to glucose. Because obesity-induced inflammation in adipose tissue has been linked to type 2 diabetes, the researchers also studied whether these compounds might have an effect on inflammation. They found that oral administration of 9-PAHSA to mice for 3 days significantly blunted the subsequent response of immune cells within adipose tissue to a potent inflammatory trigger

compared to corresponding cells from control mice that did not receive 9-PAHSA treatment. Because some of these results were from experiments in male mice, and others from experiments with females, further research could help determine whether the findings apply equally to both males and females, and whether PAHSAs may be safe and effective for treating people with or at risk for type 2 diabetes.

Yore MM, Syed I, Moraes-Vieira PM,...Kahn BB. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 159: 318-332, 2014.

UNDERSTANDING AND DIAGNOSING DIABETES COMPLICATIONS

Insights into Development of Diabetes

Complications: By studying cells derived from people with type 1 diabetes, researchers have revealed an important role in prevention of diabetes complications for a pathway that detects DNA damage and gives the cell a chance to repair it. Diabetes-related complications of the eyes, kidneys, nerves, heart, and other organs greatly affect the personal health of people with diabetes and contribute significantly to the costs of health care in the United States. Despite this toll, an understanding of the mechanisms underlying diabetes complications has remained elusive, in part due to the lack of cellular and animal models that mimic the human disease; and there is a need for novel, effective therapies. Though many people with type 1 diabetes will develop complications, valuable insights can be made from those who do not. In this research, the scientists studied “Medalists”—a group of people who have lived with type 1 diabetes for at least 50 years post-diagnosis—and compared Medalists who have experienced significant complications with Medalists who have not. The scientists reasoned that these people must have factors that protect them from

complications and that identification of these factors could help others who do develop complications.

To find these protective factors, the scientists needed to study the Medalists at a cellular and molecular level. They took advantage of a recent technological development and generated induced pluripotent stem (iPS) cells from skin cells of female and male Medalists with and without complications. These iPS cells have the potential to develop into different tissue types, allowing the scientists to study multiple tissues that are involved in diabetes complications.

Using genomic and proteomic tools, the scientists “profiled” cells from the Medalists to look for biomarkers of complications. Interestingly, genes involved in the DNA damage checkpoint pathway were suppressed in cells from Medalists with complications, but not in cells from Medalists without complications. The DNA damage checkpoint pathway monitors DNA damage and, when detected, pauses cell division to allow the cell to repair the damage. Without this checkpoint, DNA damage accumulates in a cell and can lead to cell death. The scientists found that DNA damage checkpoint pathway proteins were greatly reduced in cells from Medalists with complications, and that these cells were more likely to show signs of DNA damage.

Further investigation revealed that many of the DNA damage checkpoint pathway genes are downregulated by a small, circulating RNA molecule known as “miR200”; elevated levels of miR200 were found in cells and blood from Medalists with complications. The scientists found that increasing levels of miR200 in cells led to loss of DNA damage checkpoint control, whereas decreasing levels of miR200 in cells restored levels of the checkpoint proteins in cells from Medalists with complications. This finding is key, as it indicates that altering levels of miR200 could have value as a therapeutic approach.

The study also revealed differences between iPS cells from Medalists with complications and from Medalists without complications that may reflect other molecular pathways involved in diabetes complications. The researchers observed that the iPS cells from Medalists with complications showed significant impairment in their ability to grow and to generate different tissue types. Further research into this impairment in cell renewal may yield additional insights.

Collectively, these results revealed a role for the DNA damage checkpoint pathway in development of complications, suggested miR200 could be a therapeutic target, and demonstrated the utility of iPS cells as a cellular model to study complications and test future therapies. Additional research is necessary to determine whether targeting miR200 can protect people with type 1 diabetes from the onset and progression of diabetes complications; whether people with other forms of diabetes also get complications due to loss of the DNA damage checkpoint; and the mechanism by which type 1 diabetes can lead to elevation of miR200 in some people but not others, which could also lead to therapies tailored for individuals.

Bhatt S, Gupta MK, Khamaisi M,...Kulkarni RN. Preserved DNA damage checkpoint pathway protects against complications in long-standing type 1 diabetes. Cell Metab 22: 239-252, 2015.

For Diagnosing Diabetic Nerve Damage, the Eyes

May Have It: A noninvasive technique for imaging small nerves in the front of the eye appears to be just as effective as a skin biopsy at detecting evidence of diabetic nerve damage. Up to half of people with diabetes suffer damage to nerves throughout the body. When this damage affects both the nerves that allow feeling and those that cause movement, it can lead to decreased sensation, difficulty moving, and pain and tingling

in arms, legs, hands, and feet—a condition called diabetic sensorimotor polyneuropathy, or DSPN. Detecting and evaluating nerve damage, especially damage to the smaller nerves more often involved in early disease, is key to early diagnosis of DSPN and assessment of its progression or regression (e.g., response to clinical treatment). One test that has been used to detect small nerve fiber damage quantifies the density of these fibers in a small skin sample, usually taken from the foot or leg. While robust, however, the reliability of this technique for diagnosing DSPN remains to be thoroughly validated in large cohorts of people with diabetes, and its invasiveness makes it less useful for repeated testing.

The cornea of the eye is rich in small nerve fibers, which can be visualized and quantified using images obtained with a noninvasive laser-based imaging technique called corneal confocal microscopy (CCM). To determine whether CCM could be an alternative to skin biopsy for diagnosis of DSPN, researchers directly compared the two methods in people. Study participants included 63 men and women with type 1 diabetes who had been tested for DSPN using independent criteria, 17 of whom were diagnosed with the condition; the study also included 26 people without diabetes. Each participant underwent both the skin biopsy and CCM. Both techniques detected lower nerve fiber density in the DSPN group compared with the group without DSPN and the group without diabetes. Notably, CCM images evaluated either manually or by computer yielded similar results. While neither skin biopsy nor CCM detected 100 percent of DSPN cases, both techniques had comparable diagnostic performance. More research needs to be done in larger and more diverse groups of people with diabetes; however, these results suggest that, while both techniques are less than perfect for diagnosing DSPN, the noninvasive nature of CCM and its potential for

automation could make it preferable for use in the clinic and in clinical trials.

Chen X, Graham J, Dabbah MA, ...Malik RA. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. Diabetes Care 38: 1138-1144, 2015.

EFFECTS OF INSULIN RESISTANCE IN THE BRAIN

Linking Insulin Resistance in the Brain to

Behavioral Disorders: New studies in mice have demonstrated that insulin resistance in the brain can lead to anxiety and depression-like behaviors, suggesting a possible link between behavioral disorders and diabetes. Insulin is a hormone that plays a major role in metabolism by helping cells to absorb and use glucose (sugar) obtained from foods. Insulin resistance, a condition that occurs when cells do not respond properly to insulin, causing glucose to accumulate in the bloodstream, is a condition that often leads to type 2 diabetes. Previous research has suggested an association between diabetes and a variety of brain-related diseases and conditions, such as depression, age-related cognitive decline, and Alzheimer's disease. Insulin signaling in the brain has also been shown to be important for brain function and regulation of metabolism, but how insulin might regulate complex behavioral disorders is not well understood.

One proposed role for insulin in brain function involves the function of mitochondria, the structures in cells that unlock the energy stored in molecules like sugars and fats to make it useful to the cell. Mitochondria are also the home of two enzymes (called monoamine oxidase A and B or MAO A and B) that degrade many of the molecules nerve cells

use to signal one another. Dysregulation of these enzymes has been linked to depressive behaviors in people. To investigate how insulin activity in the brain affects mitochondria and behavior, scientists used certain genetically engineered mice (called NIRKO mice) that almost completely lack the insulin receptor in their brains. This insulin receptor deficiency disrupts signaling in the brain of NIRKO mice. At 10 months of age (“middle age” for mice), NIRKO mice are much like other mice based on body weight, food intake, blood glucose levels, and anxiety- and depressive-like behaviors. However, NIRKO mice as young as 4 months old had reduced mitochondrial function in their brains, and older NIRKO mice of both sexes displayed behavioral differences compared to controls. By 17 months of age (“older age”), NIRKO mice showed increased anxiety- and depressive-like behaviors on multiple behavioral tests, compared to their non-NIRKO counterparts. Analysis of the brains of female NIRKO mice revealed decreased signaling of the nerve cell signaling molecule, dopamine, as is often observed in human depression. Further investigation also found increased levels of MAO A and MAO B proteins in parts of the NIRKO brain, suggesting that insulin activity plays a role in signaling in both neuronal and some non-neuronal cells in the brain. Treatment of older female NIRKO mice with antidepressants that target MAO A and B virtually eliminated depressive-like behavior in one behavioral test. The scientists also found other alterations in mitochondrial structure and function. This research proposes a new explanation for how insulin resistance could affect brain function related to anxiety- and depressive-like behaviors in mice—by increasing MAO A and B activity, altering mitochondria, and decreasing dopamine activity. These findings suggest that improving brain insulin signaling may be a potential therapeutic target for the treatment of insulin resistance and diabetes-associated mood disorders. Further

research will be needed to learn if insulin plays the same role in humans and to determine if that role is similar in both women and men.

Kleinridders A, Cai W, Cappellucci L,...Kahn CR. Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. Proc Natl Acad Sci USA 112: 3463-3468, 2015.

METABOLIC REGULATORS OF HEALTH AND DISEASE

Regulating the Cell's Internal Recycling Program

During Feast and Famine: New research in mice has described interlocking regulatory pathways in the liver that are involved in important cellular responses to nutrient availability or fasting. Autophagy, a process by which cellular components are routed to the cell's “recycling centers” and broken down for use, is a key survival mechanism that can provide nutrients to maintain metabolism. Autophagy is controlled by many factors, including whether the organism has eaten lately and thus whether abundant nutrients are available to cells. When an organism fasts and nutrient levels are low, autophagy can provide raw materials and fuel to maintain the cell. Many questions remain about how this occurs, including the details of how autophagy is triggered during fasting but repressed when an organism is well-fed.

Two research groups investigating how nutrient availability regulates autophagy independently discovered that several transcriptional regulators (proteins that control whether or not genes are expressed or “turned on”) play a crucial part in this process. Using male mice as a model, both groups found that FXR, a protein in the cell nucleus that regulates various aspects of metabolism, repressed autophagy in the liver when nutrients were abundantly available. The two groups' experiments then revealed two distinct, but complementary, FXR functions. One

group determined that FXR worked in opposition to another protein involved in metabolism, PPAR α , to control the activity of genes involved in autophagy. The scientists found that FXR and PPAR α competed to bind to DNA sites that specifically regulate genes involved in autophagy. When nutrient levels were high, FXR was activated and bound to these autophagy regulatory sites, reducing the expression of autophagy-related genes. However, when nutrient levels fell during fasting, PPAR α was activated and competed with FXR for binding to the DNA regulatory sites. PPAR α binding caused autophagy genes to be expressed and autophagy to increase to help support the cell.

The other group of researchers discovered that FXR interferes with another protein that regulates gene expression. This protein, called CREB, attaches to a crucial partner protein and then binds to regulatory sites of autophagy genes to turn on the genes. When mice were well-fed, FXR disrupted the interaction between CREB and its partner, reducing autophagy. When the mice fasted, however, FXR was inactivated, and CREB regained its ability to bind to and promote expression of autophagy-related genes, triggering autophagy. The complementary findings of these two groups shed new light on the complex interactions governing the important process of autophagy. Further research is needed to clarify how FXR, PPAR α , CREB, and other proteins interact to regulate this process. If similar pathways are active in humans, future studies may reveal important therapeutic targets for metabolic disease—in which dysregulation of autophagy may alter fat storage, insulin sensitivity, and other metabolic processes—and other diseases, such as cancer and neurodegenerative diseases.

Seok S, Fu T, Choi SE,...Kemper JK. *Transcriptional regulation of autophagy by an FXR-CREB axis.* *Nature* 516: 108-111, 2014.

Lee JM, Wagner M, Xiao R,...Moore DD. *Nutrient-sensing nuclear receptors coordinate autophagy.* *Nature* 516: 112-115, 2014.

Attention to Starving Has Been Starving for

Attention: The discovery of a hormone and receptor that suppress insulin secretion during periods of fasting broadens our understanding of the intricate ways in which the body controls blood glucose (sugar) levels. During digestion, glucose from food enters the bloodstream, and insulin is secreted by the pancreas to signal cells to absorb that glucose and use it for metabolism. Hormones that signal the body to increase insulin secretion after eating—called incretins—have been the topic of considerable research, which has led to a new class of therapeutics for type 2 diabetes, called incretin mimetics. But observations in humans and other animals suggest that an opposing mechanism also exists: after a prolonged fast, the insulin-producing β (beta) cells of the pancreas are much slower to respond to an influx of nutrients by excreting insulin than they are when an individual eats more regularly. The result can be a transient elevation of blood glucose levels sometimes called “starvation diabetes,” which resolves as the β cells return to normal function. To account for this, it has been suggested that a different group of hormones—termed decretins—exists to suppress insulin release, preventing blood glucose from going too low during periods of fasting. The identities of these theorized decretin hormones and their receptors have remained a mystery. Surprisingly, the solution to the puzzle came from studying animals that do not have β cells, or even a pancreas: fruit flies.

As is typical in insects, flies respond to rising glucose levels by releasing insulin-like proteins from a group of cells in the brain. But, as in mammals, starvation results in a delayed insulin response upon re-feeding. To look for a fly decretin, investigators focused on a set of proteins that flies produce in greater abundance during starvation. They created a set of fly strains, each of which had increased levels of one of these starvation proteins, even when the flies were well-fed.

They found that the strain producing one such protein had elevated blood glucose and reduced insulin levels, suggesting the protein might be a incretin. They named it “limostatin” after Limos, the Greek goddess of starvation.

They next created flies that lack the gene encoding limostatin, and found them to have the opposite characteristics: low blood glucose and high insulin levels, as might be expected for an animal with reduced capacity to suppress insulin release. These flies also had atypically large amounts of adipose (fat) tissue, compared to normal flies, presumably because they release excessive amounts of insulin, causing too much sugar to be absorbed and stored as fat. Limostatin, the researchers went on to discover, was produced and excreted (during fasting) by certain cells of the digestive tract—and its excretion was specifically inhibited by the presence of sugars in the fly gut (rather than by fats or dietary proteins). They identified a limostatin receptor produced by insulin-producing cells, and found that flies with reduced levels of this receptor look much the same as flies lacking limostatin: they have low blood glucose and high insulin levels.

Although limostatin itself is not strikingly similar to any human proteins, the limostatin receptor is quite similar to a human protein—NMUR1—that is found on the surface of β cells, but not on other pancreatic cells. NMUR1 is the receptor for a hormone known as NMU, which is produced, they noted, in cells of the human upper intestinal tract. The researchers found that treating human β cells with purified NMU strongly inhibited insulin production, suggesting it functions in humans as limostatin does in flies—as a incretin. Interestingly, people with a very rare mutation in NMU have low blood glucose, high insulin, and early-onset obesity. These observations suggest that NMU plays an important role in inhibiting inappropriate insulin secretion in

normal, day-to-day life, not just in cases of extreme starvation. Further research may help determine whether modulating the activity of NMU or its receptors might be of clinical benefit to people with obesity or type 2 diabetes.

Alfa RW, Park S, Skelly KR,...Kim SK. Suppression of insulin production and secretion by a incretin hormone. Cell Metab 21: 323-333, 2015.

CYSTIC FIBROSIS RESEARCH

Modest Benefit Seen from Treatment for Most Common Cystic Fibrosis Mutation: New research shows that modest but significant improvements in breathing and resistance to infection can be achieved through a two-drug approach to treating the root cause of cystic fibrosis (CF) in people with the most common CF-causing mutation. CF is caused by mutations in the *CFTR* gene, which encodes a protein normally found on the membrane covering cells. This membrane controls the passage of substances into and out of the cells, and the CFTR protein contributes by serving as a channel for chloride ions. CF results when mutations inactivate both of a person's copies of the *CFTR* gene. The most common CF-causing mutation is *cftr-ΔF508*: about 90 percent of people with CF have at least one copy of this mutation, and nearly half have two copies. This mutation disrupts the chloride channel in multiple ways: it creates a channel that is unstable, that rarely gets inserted properly into the cell membrane, and that is not functional even when it is inserted. Researchers previously identified a compound, called lumacaftor, that increases the amount of $\Delta F508$ -CFTR protein that gets to the right place in the cell membrane, although its capacity to serve as a channel for chloride remains limited. A few years ago, a breakthrough medication became available for people with a different, rarer CF-causing

mutation. That mutation encodes a channel that makes it to the cell membrane but is non-functional. The new drug, called ivacaftor, works by making the non-functional CFTR channel permeable to chloride. For people with the rarer form of CF, ivacaftor greatly improved lung function and overall health.

In the new research, investigators tried a combination of lumacaftor (to increase the amount of the channel in the membrane) and ivacaftor (to allow chloride to pass through it) to treat people—both male and female—who have two copies of *cftr-ΔF508*. After 24 weeks, participants taking the drug combination had slightly better lung function than a control group taking placebo—roughly a 5 percent improvement in the amount of air they were able to exhale in 1 second. This improvement is modest, but was statistically significant. More strikingly, the participants receiving the drug combination were about one-third less likely to need to start or change antibiotics to treat symptoms of upper airway infection (a common and serious complication of CF). On the basis of these results, and because the drug combination is the first and only existing therapeutic approach that treats the underlying molecular problem in people with two copies of *cftr-ΔF508*, the

U.S. Food and Drug Administration granted expedited review and approval for its use in people 12 years of age and older with this genetic profile. It should be noted that the modest improvements conferred by lumacaftor/ivacaftor treatment do not appear to be without risk. For example, some participants receiving the drug combination experienced spikes in levels of enzymes indicative of liver injury or of damage to the heart or other muscles, and overall 4.2 percent of those taking the combination experienced adverse events that caused them to stop taking the study drugs, compared to 1.6 percent of those in the placebo group. For these reasons, it will be very important to monitor carefully the health of people taking these drugs, to limit adverse events, and to assess the long-term effects of the treatment. Research to find a drug or drug combination that is more effective and/or less prone to adverse events is ongoing; but in the meantime, there is now a therapy available that treats the underlying molecular cause of CF in about half of people with the disease.

Wainwright CE, Elborn JS, Ramsey BW, ...Boyle MP; for the TRAFFIC and TRANSPORT Study Groups. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for phe508del CFTR. N Engl J Med 373: 220-231, 2015.

NIDDK Director Testifies on Type 1 Diabetes Research

On July 15, 2015, NIDDK Director Dr. Griffin P. Rodgers testified about progress and future directions in type 1 diabetes research before the Senate Special Committee on Aging, which is led by Chairman Susan Collins (R-Maine) and Ranking Member Claire McCaskill (D-Missouri). The hearing, entitled “Diabetes Research: Improving Lives on the Path to a Cure,” was held in conjunction with the Children’s Congress, an event sponsored every 2 years by JDRF (formerly the Juvenile Diabetes Research Foundation) to highlight the value and progress of type 1 diabetes research for children and adults living with this disease.

In his testimony, Dr. Rodgers described research made possible by the *Special Statutory Funding Program for Type 1 Diabetes Research (Special Diabetes Program)*, including progress from clinical trials testing approaches to delay or prevent type 1 diabetes; recent advances toward the development of an artificial pancreas—technology to automate blood glucose sensing and insulin administration; progress on islet transplantation as a treatment approach for people with difficult-to-control type 1 diabetes; progress toward producing large quantities of insulin-producing cells in the laboratory for cell replacement therapies; and results of a comparative effectiveness clinical trial testing



NIDDK Director Dr. Griffin P. Rodgers. Photo copyright: Larry Lettera



Shown at table (left to right): Amelia Cooper, Kate Hall, Bob Amato, Dr. Griffin P. Rodgers, and Dr. Habib Zaghouni. JDRF Children's Congress delegates sit in the foreground. Photo copyright: Larry Lettera



Dr. Griffin P. Rodgers delivering testimony before the Senate Special Committee on Aging. Also shown at table (left to right): Amelia Cooper, Kate Hall, Bob Amato, and Dr. Habib Zaghouni. Photo copyright: Larry Lettera

different treatments for diabetic eye disease. The NIDDK administers the *Special Diabetes Program* on behalf of the HHS Secretary.

Testifying with Dr. Rodgers were Ms. Kate Hall, a recent high school graduate and track and field star with type 1 diabetes; Mr. Bob Amato, a former collegiate runner and coach who has had type 1 diabetes for 67 years; JDRF Children's Congress delegates Amelia Cooper, age 15, and Isabelle Levesque, age 10; and Dr. Habib Zaghouani, the J. Lavenia Edwards Chair in Pediatrics at the University of Missouri School of Medicine.

Dr. Rodgers noted in his testimony that the *Special Diabetes Program* had recently been extended through Fiscal Year 2017. The extension provides the NIDDK with an opportunity to support new and emerging research in type 1 diabetes and its complications. To solicit input on future research directions that could be supported with the new funds, the NIDDK convened a planning meeting in April 2015, which was held under the auspices of the statutory Diabetes Mellitus Interagency Coordinating Committee. At the meeting, a panel of external scientific experts and a lay representative provided input on concepts for potential new research initiatives developed by the NIDDK, other Institutes at NIH, and the Centers for Disease Control and Prevention; the panel also provided input on continuations of programs that are already supported by the *Special Diabetes Program*. Guided by that input, diabetes research strategic plans, and input that the NIDDK receives at venues such as scientific conferences and



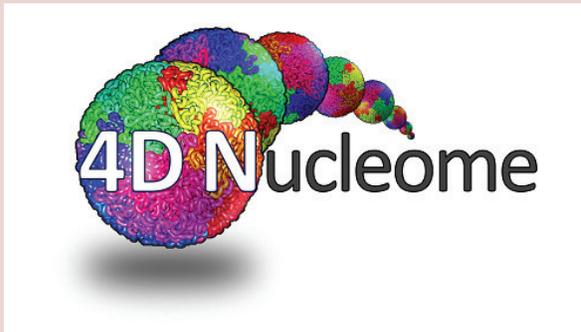
Shown at dais (left to right): Sen. Joe Donnelly (D-Indiana), Sen. Bob Casey (D-Pennsylvania), Sen. Elizabeth Warren (D-Massachusetts), Sen. Claire McCaskill (D-Missouri), Sen. Susan Collins (R-Maine), Sen. Jeanne Shaheen (D-New Hampshire), and Sen. Thom Tillis (R-North Carolina).
Photo copyright: Larry Lettera



Dr. Griffin P. Rodgers and Sen. Susan Collins (R-Maine).
Photo copyright: Larry Lettera

workshops, the Institute is identifying the most compelling areas of current research opportunity to pursue with the new funds, ensuring that the funds are used in the most efficient and scientifically productive manner possible. With the new funding, the *Special Diabetes Program* is poised to continue its exceptional track record of supporting cutting-edge type 1 diabetes research.

Exploring the Fourth Dimension on a Cellular Level: The 4D Nucleome



Imagine a stage scene in a play, with people and props bounded by the stage wings left and right, the floor front, back, and below, curtain, lights, and supports above. Now imagine that same scene, but on the tiniest of scales, with the actors now the millions of DNA base pairs in a human cell. They fill the space in three dimensions as if on winding staircases, tightly holding props of proteins for support, clustered into small groups that are organized into still larger groups we would call chromosomes. Distinct characters and activities are indicated by chemical markers costuming the DNA and proteins. All around they are bounded not by wood and curtains and lights, but by a porous yet highly structured membrane that delimits the nucleus of human and other mammalian cells. Every actor has a mark in the intricate stage directions, every costume has a meaning, every prop a purpose. But although we can see it, what principles direct staging and other choices, the architecture of this molecular level scene? And what of when the scene is no longer still, and we enter time—the fourth dimension—and the play moves forward? The actors move, in smaller and larger groups; they interact, change

costumes, change props—but what if they stumble and fall, ignore cues, lose props, drop or misdirect lines, miss marks? How does the play change—and thus, the life of a cell, a tissue, a whole organism? Scientists involved in an ambitious new NIH program called the 4D Nucleome are seeking answers to these questions and more.

The 4D Nucleome Program is supported by the NIH Common Fund. Established in 2006 and administered through the NIH Division of Program Coordination, Planning, and Strategic Initiatives, the purpose of the Common Fund is to develop and invest in scientific programs that are transformative and have clear goals and deliverables within a specific time frame. These programs are also trans-NIH in nature and scope, transcending the missions of individual Institutes as they benefit the scientific enterprise. Focused on the cell's nucleus, in which chromosomes—the individual structures containing the genetic blueprint for a cell—reside, the 4D Nucleome Program aims to understand the principles underlying nuclear organization in space and time, the role nuclear organization plays in gene expression—how genes are “turned on” or “turned off”—and cellular function, and how changes in nuclear organization affect normal development as well as various diseases.

To establish the 4D Nucleome Program, in 2014 the NIH issued six related Funding Opportunity Announcements, each representing a core research or research support initiative important

to the overall goal of the program. In October 2015, the NIH announced that the 4D Nucleome Program is supporting its first set of 29 awards under these six core initiatives, totaling approximately \$25 million. The program includes support for an interdisciplinary consortium to explore nuclear organization and function; development of new chemical, biochemical, and imaging tools; studies of structural and functional subregions within the nucleus; an organizational hub to facilitate collaboration and resource sharing; and a data center to coordinate and integrate data generated by the 4D Nucleome investigators.

One of the core initiatives, the Nuclear Organization and Function Interdisciplinary Consortium, is being administered by the NIDDK. The NIDDK has taken a leadership role in the 4D Nucleome Program because complex diseases and conditions, such as diabetes and metabolic syndrome, are suspected to involve disruptions in normal nuclear architecture. For example, many genome-wide association studies have been performed in recent years to better understand the genetic basis for individual predisposition to

diabetes. When analyzed as an aggregate, these studies reveal the existence of over a hundred genetic variations associated with diabetes, with approximately 90 percent of them located in non-coding regions of the genome. These genetic variants likely contribute to disease by disrupting genome architecture and/or the ability of regulatory elements within the nucleus to properly control the spatial and temporal expression of genes involved in metabolic functions. It is also known that the activity of genes involved in metabolism is affected by circadian clocks—central mechanisms that allow most organisms to coordinate biological functions and behavior with environmental changes in light and dark cycles. Evidence suggests that there are regular spatial as well as functional changes in circadian clock genes within the nucleus during specific time periods, and hence a dynamic point of vulnerability to mishaps that could, in turn, affect metabolic gene expression. Thus, information from the 4D Nucleome efforts could be highly beneficial to a better understanding of metabolic and other diseases within the NIDDK mission, and the discovery of novel drug targets.

SGLT2 Inhibitors: Harnessing the Kidneys To Help Treat Diabetes

Existing treatments are helping many people with diabetes live healthier lives, but there is still an urgent demand for new diabetes medications. Many years of research have enhanced understanding of diabetes and its effects on the body, including its role in kidney damage. Likewise, research into how the kidneys function has led to a better understanding of how the kidney manages glucose (sugar) and fluid in the body. Collectively, this research has led to the discovery of a new class of drugs that targets the kidneys to help control blood glucose in people with diabetes.

Glucose is a sugar that serves as the body's chief energy source. For those with diabetes, their cells have difficulty using glucose properly, leading to hyperglycemia (high blood glucose). Some people with type 2 diabetes can control their condition with physical activity and diet, while others require diabetes medications. As the disease progresses, many require injections of insulin, a hormone which helps the body utilize glucose. Existing diabetes drugs can help people with diabetes maintain their blood glucose levels in a healthy range, reducing their chances of complications later in life. However, these existing treatments sometimes carry side effects (such as hypoglycemia, or low blood glucose) and/or restrictions that can limit their usefulness. Moreover,

even with the expanded choice of treatments now available, meeting recommended blood glucose level targets can be challenging.

From many years of dedicated research, a new approach to reducing blood glucose levels has emerged: a new class of diabetes drugs, called SGLT2 inhibitors, that allows the kidneys to dispose of excess blood glucose in the urine. Clinical studies in people with type 2 diabetes have shown that these medications can safely and effectively lower blood glucose levels and improve glycemic control.

The path of discovery from basic research to effective, U.S. Food and Drug Administration (FDA)-approved diabetes drugs was paved with decades of work by many scientists, including NIDDK-supported researchers. The SGLT2 inhibitors are a prime example of how discovery research into how the body works can result in new disease treatments.

The Kidneys and Diabetes

Every day, a healthy adult's two kidneys, each about the size of a fist, together filter 120 to 150 quarts of blood. Blood carrying wastes enters the kidneys, and the kidneys' millions of filtering units, called nephrons, filter that blood in a two-step process. First, blood passes through

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the glomerulus, a structure which keeps blood cells and larger molecules, such as proteins, in the blood, while allowing wastes and excess fluid to pass through. The filtered fluid then passes through the tubule, which reclaims needed minerals and glucose, sending them back to the bloodstream. Wastes and extra fluid continue on to the bladder as urine. In this way, the kidneys maintain blood's healthy composition, keep levels of electrolytes such as sodium and potassium stable, and (through fluid management) contribute to healthy blood pressure.

The kidneys play an important role in managing glucose levels in the body. Because glucose is small enough that it can pass through the glomerulus, it will end up in the urine if it is not reclaimed or "reabsorbed." Because the body uses glucose as fuel, losing significant amounts of glucose in the urine would be wasteful for a healthy person. To prevent this loss, healthy kidneys in people without diabetes recapture virtually all the filtered glucose and return it to the bloodstream.

After the blood is filtered through the glomerulus, the filtered fluid (or "filtrate") moves on to the tubule, where the business of glucose reabsorption takes place. In the tubule wall, one side of each cell faces the filtrate and the other faces the circulation. In this way, tubule cells can act as both sensors monitoring the components of the filtrate, and conduits that can move materials from the filtrate back into the blood. The filtrate flows over the tubule cells, and transport proteins on the tubule cell surface recapture the glucose, much like workers plucking items from a conveyor belt.

Glucose is transported into the tubule cells and then pumped out the other side, back into the blood.

However, the kidneys' glucose reabsorption system is optimized to work best when blood glucose concentrations are in a normal range. In people with poorly controlled diabetes, who have increased blood glucose levels, this system begins to break down. The amount of glucose in the blood exceeds the kidneys' ability to recapture it, and some glucose continues through the tubules and is lost in the urine, a condition called glucosuria.

The Identification of SGLTs—Novel Proteins That Transport Glucose in the Kidney and Other Tissues

For many years, the exact details of how kidney cells reabsorb glucose were unknown. The first clue as to how the kidneys accomplish this task was discovered in the early 1980s by NIH-supported researchers who noticed differences in glucose transport capacity throughout the rat kidney tubule: the early part of the tubule could absorb more glucose more quickly than the downstream part of the tubule. Understanding of how this worked on the molecular level emerged from studies of how glucose from food is absorbed by the cells lining the intestine. NIDDK-supported researchers studying the cells lining the intestine discovered the gene for the intestinal glucose transport protein. The protein belonged to a new class of glucose transporters called sodium-glucose cotransporters, or SGLTs. The intestinal transport protein was named SGLT1. Scientists then found a second, closely related

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protein, SGLT2. Both SGLT1 and SGLT2 are responsible for glucose transport in the kidney.

SGLT1 and SGLT2 are proteins on the cell surface of the tubule cells. Both reclaim glucose from the kidney filtrate, moving glucose together with sodium into the tubule cells, where they can then be returned to the blood. SGLT2 is found earlier in the tubule and is a very high-capacity glucose transporter, while SGLT1 is found later in the tubule and is a lower-capacity transporter. Thus, filtered glucose will first encounter SGLT2 before encountering SGLT1. SGLT2 is responsible for 90 percent of the total glucose absorption as urine is made, while SGLT1 is responsible for the remaining 10 percent. In addition to the kidney and intestine, SGLT1 is also found in many other tissues of the body.

Because of their key role in glucose reabsorption, the SGLTs, particularly SGLT2, were promising drug targets to alter blood glucose levels. Healthy kidneys can reabsorb up to 180 grams (roughly 0.40 pounds) of glucose per day. If a medication could safely block SGLT2 activity and encourage the kidneys to pass that glucose out with the urine rather than reclaim it back into the blood, that might be an elegant solution to persistently high blood glucose levels. In fact, a condition called familial renal glucosuria (FRG) already demonstrated this approach in nature. This condition is caused by changes in the gene coding for SGLT2, resulting in reduction in SGLT2 activity. This reduced activity prevents most glucose in the filtrate from being reclaimed, and people with FRG lose significant amounts of glucose in the urine. Interestingly, for reasons that

are not entirely understood, this condition does not seem to cause hypoglycemia or any serious side effects. Therefore, researchers asked, could SGLT2 inhibitors be safe and effective for use in people with diabetes?

Treating Diabetes with the Help of the Kidneys

By the time the SGLT proteins were discovered, an SGLT inhibitor called phlorizin had been studied for over 150 years, although only in recent decades have scientists discovered its mechanism of action. Phlorizin came from the root bark of the apple tree. As early as 1933, it was briefly tested in a very small number of people, and scientists found that it could increase glucose in the urine, lower blood glucose levels, and prevent reabsorption of glucose. However, its effects were not limited to the kidney. Because it inhibited glucose absorption in the intestine, was poorly absorbed when taken orally, and interfered with glucose transport in other parts of the body, it was not suitable for use in people. Nonetheless, studies of phlorizin were important to understanding how sodium-glucose transporters worked, and scientists suspected that it might inhibit the SGLTs. Indeed, in 1995, NIDDK-funded researchers found that phlorizin inhibited both SGLT1 and SGLT2. Because SGLT1 is found in many tissues and plays a key role in absorbing glucose in the intestine, this explains some of phlorizin's side effects.

As more became known about phlorizin and SGLTs, scientists became interested in using phlorizin as a starting point to develop a treatment

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for diabetes. Work over the next few decades focused on developing phlorizin derivatives that were more potent, more specific to SGLT2, and that lasted longer in the bloodstream. This research resulted in the discovery and testing of improved SGLT2 inhibitors.

The SGLT2 inhibitors have been extensively studied in industry-supported clinical trials and were found to be safe and effective at improving glucose control in adults with type 2 diabetes. This research has culminated in FDA approval of several drugs for treatment of type 2 diabetes. The first SGLT2 inhibitor to be FDA-approved was canagliflozin (marketed as Invokana®) in March 2013, followed by the approval of dapagliflozin (marketed as Farxiga®) in January 2014 and empagliflozin (marketed as Jardiance®) in August 2014. These medications provide new tools to help control blood glucose levels in adults with type 2 diabetes.

The approved SGLT2 inhibitors are effective in reducing hemoglobin A1c (HbA1c), a measure of blood glucose levels. SGLT2 inhibitors can be used with other oral or injectable diabetes medications, including insulin. This is important because diabetes is a progressive disease which often requires additional medicines over time as insulin production decreases. Another advantage of SGLT2 inhibitors is that they do not cause hypoglycemia (low blood sugar) in the absence of other drugs with this side effect. Because sodium, like glucose, is reabsorbed by the SGLTs, SGLT inhibition also increases loss of sodium in the urine. SGLT2 inhibitors can cause a modest reduction in systolic blood pressure, but because this effect is so small it may not be clinically

meaningful, and these drugs are not indicated for the control of blood pressure.

One recent industry-supported clinical trial found that people with type 2 diabetes and cardiovascular disease had a lower rate of death from cardiovascular causes when they added the SGLT2 inhibitor empagliflozin to their standard care. Cardiovascular death was reduced by 38 percent, although there was no significant effect on nonfatal heart attacks or strokes. Of note, the study was limited to participants with established cardiovascular disease and a previous cardiovascular event such as a heart attack or stroke. Also, most participants were older (average age 63) and had long-standing diabetes (57 percent had diabetes for more than 10 years). Studies are ongoing to determine the effects of other SGLT2 inhibitors on cardiovascular disease. More research is also needed to determine the effect of empagliflozin on cardiovascular disease in the broader population with diabetes—those who are younger, have shorter durations of diabetes, and do not have pre-existing cardiovascular disease. Research is also needed to understand whether the reduction in cardiovascular death was due to reduced blood pressure, lower fluid volume, or other mechanisms.

SGLT2 inhibitor use does have some restrictions and side effects, however. The glucose-lowering action of these drugs is dependent on adequately functioning kidneys. People should not take SGLT2 inhibitors if they have severely impaired kidney function. Some of the SGLT2 inhibitors are not effective and may have more side effects in

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people with moderate kidney dysfunction. SGLT2 inhibitors are also not approved to treat patients with type 1 diabetes. Because SGLT2 inhibitors are diuretics, common side effects include increased thirst and urination. People taking SGLT2 inhibitors may develop low blood pressure when going from lying down or sitting to standing. Older people are particularly susceptible to this drop in blood pressure, called orthostatic hypotension, which can cause dizziness and falls. People taking SGLT2 inhibitors also have an increased risk for genital fungal infections (such as yeast infections in women) and urinary tract infections. At least one SGLT2 inhibitor (canagliflozin) increases both the risk of loss of bone density in the hip and lower spine and the risk of bone fractures. The FDA has warned that fractures can occur as early as 12 weeks after starting this drug and with only minor trauma. Because these drugs impact mineral metabolism and may increase the risk of falls, health care providers should be vigilant regarding bone health. There have also been reports that these medications may cause ketoacidosis, a potentially dangerous metabolic condition, and the FDA has warned patients and caregivers to be alert for the signs and symptoms of this condition. Diabetic ketoacidosis is usually seen only with very high blood glucose levels, but ketoacidosis has been reported to occur with only mild or moderately increased glucose in people taking SGLT2 inhibitors. This side effect may be more common in people taking insulin who reduce their insulin dose, and in those with acute illness, infection, alcohol use, or reduced food and fluid intake.

The Future of SGLT Inhibitors

Building on the successful use of existing SGLT2 inhibitors in people with type 2 diabetes, more research is being done on this class of medications. SGLT2 inhibitors provide a significant reduction in blood glucose levels, but they do not reduce blood glucose to healthy levels in all people who have been given these medications. Thus, some compounds that inhibit both SGLT2 and SGLT1 are under investigation to increase glucosuria even more than can be achieved with SGLT2 inhibitors alone. Additionally, several new SGLT2-specific inhibitors are in pre-clinical development, and some have been approved for type 2 diabetes treatment in other countries. Ongoing studies will provide information about whether SGLT2 inhibitors are safe and effective in people with type 1 diabetes.

After drugs receive FDA approval, new information about risks and benefits often emerges as more people receive the drug. As described above, the SGLT2 inhibitors' risks and benefits will require further study. More research will determine the magnitude of the risks (such as bone fractures) and benefits (such as reductions in cardiovascular-related deaths), in which patients they occur, and whether these effects are specific to certain drugs or are common to all SGLT2 inhibitors. The FDA continues to work closely with manufacturers to monitor emerging information about the safety of the three drugs in the SGLT2 inhibitor class that have now been approved and to alert caregivers and patients to the latest information.

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Better Treatments Through Research

Diabetes is a costly, chronic disease that can be difficult to manage effectively. People with diabetes can find it difficult to keep their blood glucose levels within a healthy range, and new medications that help them achieve these goals are needed. SGLT2 inhibitors, a new class of medications for the treatment of diabetes, are the result of decades of dedicated research by many scientists across the globe. The development of the SGLT2 inhibitors built upon years of research into how the kidneys function, and their story is a wonderful example of how research in one area can lead to advances in

another. The NIDDK and the NIH have supported many stages of this research: basic inquiries into how the kidneys function, discovery of the SGLT proteins, pre-clinical development and testing of SGLT inhibitory compounds, and elucidation of how SGLT2 inhibitors work to help those with diabetes meet their health goals. Until recently, people with type 2 diabetes had only a few classes of drugs to choose from when diet and exercise were not sufficient to control their blood glucose. Basic research expanding our knowledge of how the body works has paid off by laying a firm foundation for the discovery of new medicines that are helping people with diabetes build a healthy future.

Dr. Jean E. Schaffer— An Unexpected Role for Certain Small RNAs in Diabetic Complications: sno in the Forecast

Dr. Jean Schaffer is the Virginia Minnich Distinguished Professor of Medicine, and Director of the Diabetic Cardiovascular Disease Center and Diabetes Research Center at Washington University School of Medicine, St. Louis, Missouri. She earned her M.D. from Harvard Medical School, where she also served as a resident and intern at the Brigham & Women's Hospital. She was a Clinical and Research Fellow in Cardiology at Beth Israel Hospital, and a Post-doctoral Fellow at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology. Her laboratory seeks to understand the physiological links between dyslipidemia and heart disease in people with diabetes and obesity. Dr. Schaffer described some of her laboratory's research studies at the January 2015 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, of which she is a member.

Many of the heaviest burdens of diabetes—death, disability, and financial cost—stem from its complications, rather than from the disease itself. In particular, the cardiovascular complications of diabetes, which include heart attack, stroke, and heart failure (a dangerous reduction in the heart's

ability to pump blood through the circulatory system), are the leading causes of death among people with diabetes. What are the biological links between diabetes and cardiovascular disease? And, other than treating or preventing diabetes in the first place, how might we intervene to prevent the cardiac complications of diabetes? Through the use of genetic screens in cultured cells and the study of mouse models, Dr. Schaffer and her colleagues have discovered one such potential intervention point: a group of molecules, designated small nucleolar RNAs, or snoRNAs, that appear to play an important role in tissue damage from metabolic stress.

Fatty Acids as Drivers of Cardiac Complications of Diabetes

"Dyslipidemia"—abnormally high levels of harmful lipids (fats) and/or low levels of beneficial lipids—is a common problem in Americans, especially those who are overweight or obese. The insulin resistance that often leads to type 2 diabetes has also been shown to promote dyslipidemia by driving the liver to increase production of certain lipids and by causing the failure of fat

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tissue to appropriately regulate the release of lipids. Abnormalities in lipid metabolism are also seen in type 1 diabetes. Dr. Schaffer described how elevated blood levels of free fatty acids can induce damage to the heart muscle and lead to heart failure in people with diabetes, even in the absence of underlying blockages in the blood vessels of the heart.

A healthy heart muscle has limited capacity to store the energy it needs for its ceaseless, life-long task of pumping blood through the circulatory system. It has therefore evolved a tremendous capacity to scavenge calorie-containing molecules from the blood supply, including lipids. In healthy individuals—those without dyslipidemia—about two-thirds of the heart's caloric needs are fulfilled by its uptake of free fatty acids from the blood. In the setting of dyslipidemia, the diabetic heart takes up fatty acids to an even greater extent, but not all of this lipid can be metabolized by the heart muscle cells. Dr. Schaffer noted that work from others has shown that people with diabetic heart failure tend to have abnormal deposits of fat within the muscle cells of the heart. She speculated that in people with diabetes, increased absorption of free fatty acids due to dyslipidemia might lead not only to these deposits, but also to the functional defects seen in diabetic heart failure.

To test this hypothesis, she and her colleagues generated and analyzed a strain of mice whose hearts absorb these fats even more efficiently than normal. This has the effect of promoting accumulation of fat deposits in the heart muscle cells of these animals. They found that the hearts

of these animals also rapidly develop features of diabetic heart failure. Further, as is sometimes observed in other tissues where excessive amounts of various metabolites occur in disease, they saw evidence that these accumulating lipids were triggering formation of “reactive oxygen species,” which are types of molecules that can cause cell damage or even cell death if they become abundant enough. Thus, these results support a model in which increased deposition of lipid in the heart muscle can have damaging effects.

A Large Role for Small Nucleolar RNAs

To identify novel ways to treat or prevent heart failure and other diabetic complications, Dr. Schaffer and colleagues used a rodent cell line to look for mutations that would allow the cells to withstand what are normally toxic levels of free fatty acids and glucose (sugar). In this way, they identified a mutation that inactivated a gene called *Rpl13a*. Like other genes in mice and humans, the *Rpl13a* sequence is interrupted by sections of DNA called introns that do not encode portions of the Rpl13a protein. To utilize the gene to make the Rpl13a protein, the cell produces an RNA version of the gene, called the gene's transcript, from which the introns are then removed. However, while the introns of most genes are rapidly destroyed following removal from the initial transcript, four short sections of the *Rpl13a* introns, called small nucleolar RNAs (snRNAs), have a function of their own, and are retained.

Because the mutation disrupted production of both the Rpl13a protein and the snRNAs,

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an important question became whether it was elimination of the protein that conferred resistance to toxic lipid levels (lipotoxicity), or was it the elimination of one or more of the snoRNAs? To answer this question, Dr. Schaffer's group first used genetic complementation to identify the critical regions of *Rpl13a* that could restore sensitivity to metabolic stress in the mutant cells. The genomic region was sufficient to complement the mutant, but only if the snoRNAs were intact. In a second approach they showed that knocking down the snoRNAs, but not the Rpl13a protein, rendered wild type cells resistant to lipotoxic stress. These findings point to a key role for the snoRNAs in the deleterious effects of high lipid levels.

Nucleolar RNAs That Can Leave the Nucleolus

snoRNAs are generally found within the nucleolus, a large structure within the cell's nucleus that produces ribosomes, molecular machines that perform the critical role of translating RNA from genes into proteins. As expected, Dr. Schaffer and colleagues found that *Rpl13a* snoRNAs are found in the nucleolus under normal conditions. Interestingly, however, when cells are subjected to unhealthy levels of fatty acids, the *Rpl13a* snoRNAs exit both the nucleolus and the nucleus and accumulate in the cytoplasm (*i.e.*, outside the nucleus but still inside the cell).

Dr. Schaffer's group observed this unusual location for the *Rpl13a* snoRNAs not only when cellular fatty acid levels rise, but also following other triggers of

reactive oxygen species. It is unknown what these snoRNAs might be doing outside of the nucleolus, but the data suggest they may be involved in destroying cells damaged by reactive oxygen species, leading to tissue damage. Indeed, using a technique that selectively reduced levels of the *Rpl13a* snoRNAs in the cell, they found they could increase the cell's resistance to these toxic molecules. Similarly, they found that reducing the levels of snoRNAs in the liver of a mouse helped protect the animal from oxidative stress. This suggests that the snoRNAs may play an important role in response to many environmental stimuli that damage tissues through reactive oxygen species.

To better understand the physiological function of the *Rpl13a* snoRNAs, they recently created mice that lacked these non-coding RNAs entirely. (That is, with a version of the *Rpl13a* gene that encodes the Rpl13a protein, but not the snoRNAs.) Dr. Schaffer's group is testing how these animals respond to different types of metabolic stress. These "sno-less" mice will provide a powerful new tool for understanding the damaging effects of ectopic lipid accumulation in tissues like the heart. They may also provide new insights into mechanisms that underlie other diabetic complications that result from excess metabolites.

Further testing will be needed to determine whether the corresponding human snoRNAs promote diabetic heart disease. If they do, it may one day be possible to improve treatment of diabetes by blunting their impact through the development of therapies that lower snoRNA levels.

Bariatric Surgery Offers Hope as a Treatment for Some People with Type 2 Diabetes

Several years ago, when Karen Voll learned that she had been accepted as a participant in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded Triabetes research study and would receive bariatric surgery, she was ecstatic. “That was the happiest day,” she laughs, “I can still remember that day!” The Triabetes clinical trial aims to understand the health benefits and risks of bariatric surgery in people who have mild or moderate obesity along with type 2 diabetes that has been particularly difficult to control by other means. Soon after an evaluation showed that it could be an appropriate treatment option for Karen, she underwent Roux-en-Y gastric bypass surgery, and the health improvements that she experienced were immediate and dramatic. Her previously uncontrolled type 2 diabetes was completely reversed, even without any medications.

A Difficult Diagnosis

The relief and hope that Karen felt when she first joined the Triabetes study were so strong because she was no stranger to type 2 diabetes and its health consequences. Several members of her family are either living with the disease, or have succumbed to its complications. That’s why, in

2005, when she was diagnosed with the disease in her late forties, she had a clear idea of what this difficult news meant. Following her diagnosis, she began taking the type 2 diabetes medication metformin and tried unsuccessfully to lose weight, as she was mildly obese. Despite her best efforts to control her blood (glucose) sugar levels, her health deteriorated. “I just felt sick all the time,” she remembers, “some days I didn’t even feel like crawling out of bed.” This was a challenging time, considering Karen worked a full-time job in addition to helping with her husband’s electrical contractor business. As she describes it, she “constantly just didn’t feel right at all. Just felt off.”

Over the years following her diagnosis, Karen’s health continued to decline. During that time, she remembered witnessing the progressive deterioration in health of her mother-in-law and her own father, both of whom died as a result of type 2 diabetes. It “just scared me to death,” Karen recalls. These memories filled her with a determination to find a way to manage her diabetes.

Karen knew about bariatric surgery as a weight-loss treatment; her husband, who had more severe obesity and also suffered from type 2 diabetes, had bariatric surgery 10 years earlier. However,

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her BMI (body mass index, a measure of weight relative to height) was in a range that is considered mildly obese. By contrast, guidelines generally recommend bariatric surgery only for patients with higher levels of obesity, and particularly for patients with severe obesity. Through her own research on the Internet, she learned of a study, called “The Triabetes Study: A Trial to Compare Surgical and Medical Treatments for Type 2 Diabetes,”

that was being conducted in nearby Pittsburgh, Pennsylvania, by a group led by Dr. Anita Courcoulas, a bariatric surgeon. The Triabetes study was enrolling participants

with BMIs in the range of mild obesity whose type 2 diabetes was particularly difficult to control. Karen felt she might be a good candidate, so she contacted Dr. Courcoulas' office to see if she was eligible to participate in the clinical trial. After consultations with staff associated with the research study (including a dietician, psychologist, and physician), she learned the great news that she was a good fit.

Roux-en-Y Gastric Bypass Surgery and the Triabetes Study

Previous research had shown that, in people with severe obesity (a BMI of 40 or higher), bariatric

surgical procedures can have dramatic benefits, such as significant and sustained weight loss, improved control of blood sugar levels, and even reversal (remission) of type 2 diabetes. However, there has been little scientific evidence to define the risks and benefits of bariatric surgery for people with lower levels of obesity, and particularly for people with mild obesity, who suffer from uncontrolled type 2 diabetes. (For a

woman of average height, about 5 feet 4 inches tall, a BMI of 30, or mild obesity, would correspond to a body weight of 175 pounds; and a BMI of 40, severe obesity, would correspond

to a weight of 233 pounds.) Among individuals with severe obesity who experience remission of their type 2 diabetes after bariatric surgery, some find that their diabetes subsequently recurs. However, the longer-term health effects of bariatric surgery have not been well studied, and for people with milder levels of obesity, there was limited data even on shorter-term outcomes.

To begin addressing the important question of the effects of bariatric surgery in people with type 2 diabetes and lower levels of obesity, like Karen, the Triabetes clinical trial compared two different bariatric surgery procedures—Roux-en-Y gastric bypass (RYGB) and laparoscopic

When Karen learned that she had been accepted as a participant in the NIDDK-funded Triabetes research study and would receive bariatric surgery, she was ecstatic. “That was the happiest day,” she laughs, “I can still remember that day!” The Triabetes clinical trial aims to understand the health benefits and risks of bariatric surgery in people who have mild or moderate obesity along with type 2 diabetes that has been particularly difficult to control by other means.

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adjustable gastric band (LAGB)—with an intensive lifestyle weight loss intervention. The goal was to determine each intervention's relative effectiveness at reducing weight and improving blood sugar levels. Karen and the other volunteers were randomly assigned to receive one of these three treatments, and the researchers then evaluated their health outcomes.

In 2011, Karen was randomly assigned to receive RYGB surgery. The most commonly performed procedure at this time, RYGB reduces the size of the stomach and connects the upper part of the stomach to the lower part of the small intestine, so that food bypasses a large portion of the gastrointestinal tract in which digestion and nutrient absorption normally take place.

Maintaining Health Benefits Through Lifestyle Changes

Karen began feeling health benefits soon after the RYGB procedure. Remarkably, her diabetes was completely reversed within weeks after surgery. She no longer required insulin or metformin to manage her blood sugar levels. "Three weeks after I had the surgery," she remembers, "I was no longer on the insulin. My numbers were perfect. I no longer had high cholesterol. I no longer had high blood pressure. I was off all that medication.

Within 3 weeks!" Within 6 months, her weight fell dramatically, and continued to drop for the following 6 months. Her weight then increased slightly and stabilized at a level well within the healthy range for her height.

The first weeks and months following surgery were challenging nonetheless. "The first few weeks, you're on pure liquids," she recalls about her initial diet, "then you start slowly introducing solids."

Dieticians associated with the Triabetes trial

provided clear recommendations for her diet moving forward (e.g., lean proteins, vegetables, fiber), which she

has been following quite closely over the years. Unexpectedly, Karen's taste preferences seemed to change after the surgery. She no longer craves certain foods that were a regular part of her diet. "I'm Italian, so pasta was a big thing for us, that we grew up on," Karen reminisces. But now, she doesn't have the desire for pasta that she once had. "My taste buds have completely changed. I don't even think about it now." A few staple dishes now constitute the bulk of her daily dietary routine, such as salads, homemade chicken soup (without noodles), and tuna fish, and she eats only small quantities at a time.

In addition to diet, Triabetes staff recommended at least 200 minutes of exercise per week, working up to 300 minutes. Due to a back problem,

"Three weeks after I had the surgery," Karen remembers, "I was no longer on the insulin. My numbers were perfect. I no longer had high cholesterol. I no longer had high blood pressure. I was off all that medication. Within 3 weeks!"

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Karen has not been able to engage in strenuous physical activity, but she walks “at a pretty good pace” for 30 to 45 minutes, 5 days a week. In addition to walking, she is very active in various other aspects of her life, including mowing her lawn and gardening. “I love doing my gardening...babying my tomatoes right now!”

The health benefits and weight loss associated with bariatric surgery have given Karen a level of energy that she simply did not have before. “I know I’m a lot happier now, I’m a lot healthier. I feel better. I have more energy to do things. Before, I could hardly walk up and down steps; now I can run up...to my second floor. I can run up those steps with no problem at all.”

Like Karen, many other participants in the Triabetes study also experienced health benefits. Evaluating outcomes 12 months post-surgery, the researchers found that RYGB was more effective than LAGB for weight loss and improved control of type 2 diabetes. Both of these surgical treatments were significantly more effective than lifestyle interventions alone for this group of individuals. Importantly, however, only a few of the participants who received RYGB surgery experienced complete remission of type 2 diabetes, as Karen did. Those with complete remission of type 2 diabetes had normal

blood sugar levels without need for diabetes medications. Half of the participants who received RYGB experienced partial remission: their blood sugar levels, although above normal, were no longer in the range of diabetes, and they were able to discontinue their diabetes medications. None of the participants in the original lifestyle treatment group experienced complete or partial diabetes remission.

Karen and others continued participating in the study, so that the researchers could gather

data on their health outcomes several years after surgery. All of the participants, including those who originally

received surgery, were given lifestyle instruction on weight-control behaviors, with the hope that it would help them maintain their weight loss. Among those who had received RYGB, the overall rate of diabetes remission (partial or complete) was 60 percent 1 year after surgery, but by 3 years after surgery, fewer people (40 percent of the participants) had the benefit of diabetes remission. In the LAGB group, the rate of complete or partial diabetes remission remained stable at 29 percent at the two time periods. A few individuals in the surgical groups experienced complications (such as needing another surgery, ulcers, kidney stones, or hospitalization for dehydration), but Karen did not.

“I know I’m a lot happier now, I’m a lot healthier. I feel better. I have more energy to do things. Before, I could hardly walk up and down steps; now I can run up...to my second floor. I can run up those steps with no problem at all.”

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The Triabetes study was relatively small, with only about 20 participants in each group. Nonetheless, the study yielded important knowledge about the health outcomes from bariatric surgery, and useful information to help researchers plan future studies on longer-term risks and benefits. For example, based on the study's findings of

differences among the participants in diabetes remission and surgical complications, future research could begin to

provide important insights about which people are likely to benefit from bariatric surgery, like Karen did, and which potential patients might not achieve remission of their diabetes, or might experience complications. This research could help inform treatment decisions. Longer-term studies are also necessary because type 2 diabetes can recur even after surgery. Type 2 diabetes is a disease that progresses over decades, and it remains unclear how long the effects of bariatric surgery will last for different individuals. Additionally, people who have undergone gastric bypass surgery need lifetime health monitoring to help avoid nutritional deficiencies. Because only a few of the participants, like Karen, experienced complete diabetes remission, future research could also yield insights that might further increase benefits for people who choose surgery.

Longer-term studies are also necessary because type 2 diabetes can recur even after surgery. Type 2 diabetes is a disease that progresses over decades, and it remains unclear how long the effects of bariatric surgery will last for different individuals.

Life After Bariatric Surgery—Continuing Health Benefits Four Years Later

Now, 4 years after her bariatric surgery, Karen remains at a healthy weight and free from diabetes and high blood pressure. She is grateful for the support of her family, which has been

helpful in her adherence to a healthful lifestyle. Because her husband previously had RYGB surgery, she was well aware of the associated

challenges and lifestyle changes. Unlike Karen, he did experience some complications, but overall he has been able to maintain his healthier weight. "My husband...was my number one cheerleader," she says.

Karen continues to have monthly follow-up phone calls with Triabetes study staff, and annual visits to Dr. Courcoulas' office. In addition to providing valuable longer-term data for the study, these communications and interactions ensure that she can receive regular guidance should any problems arise. But thankfully, the lifestyle changes that she adopted following her surgery have successfully led to sustained health.

Despite the fact that Karen was only mildly obese prior to her surgery, her uncontrolled type 2 diabetes forced her to consider all

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treatment options. Based on the Triabetes and other studies, bariatric surgery appears promising as a treatment for some people with type 2 diabetes and milder levels of obesity, along with those who have severe obesity. Dr. Courcoulas and other scientists caution that it is important to build on these results and evaluate

longer-term outcomes in more people. Bariatric surgery is more invasive than other intervention strategies and thus comes with some inherent risk—but in Karen's case, it has been very effective while other strategies were unsuccessful. She says, "I've been asked, 'Would I do it again?' And I've said, 'in a heartbeat I would.'"

Improved Diagnosis Leads to an Easier Treatment for Monogenic Diabetes



Lilly (center) with father, Mike (left), and mother, Laurie (right)

Sixteen-year-old Lilly remembers the pressure of being a young child on insulin therapy for what was thought to be type 1 diabetes—the monitoring of her blood sugar (glucose), the pain of needle sticks, the lack of independence and freedom. “It was very stressful,” Lilly says, “and it was really hard.”

But now, instead of shots and constant monitoring, Lilly only needs to take several pills twice a day.

This new treatment is possible because Lilly actually has a rare—and often misdiagnosed—

form of diabetes known as monogenic diabetes, and some types of monogenic diabetes can be treated with a class of drugs called sulfonylureas. This medication helps her pancreas release the

insulin it makes, allowing her to live without the need for insulin injections.

Lilly’s mother, Laurie, describes Lilly’s transition from insulin to sulfonylurea as a miracle. “We prayed for a long time after Lilly was diagnosed that there would be a cure,...but we in our wildest dreams didn’t think that it would happen so quickly, and certainly not that it would come in the form of a pill!”

Growing Up with Diabetes

Lilly was diagnosed with type 1 diabetes when she was 1 month old. Type 1 diabetes is an autoimmune disease in which the body launches a mistaken attack that destroys the insulin-producing beta cells in the pancreas. As a result, people with this disease must carefully monitor their blood sugar levels and must receive insulin either by injection or through an insulin pump. Lilly’s pancreas was not releasing insulin, though her case was atypical because tests indicated that her immune system wasn’t

attacking her beta cells. Additionally, though type 1 diabetes is most often

diagnosed in young people, it is not often found in month-old babies. However, as Laurie says, the doctors diagnosed Lilly with type 1 diabetes because “there was nothing else to call it.”

“You can never let your guard down when you have a child with diabetes, let alone a baby with diabetes,” Laurie says.

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Lilly's parents provided the rigorous monitoring and treatment Lilly needed. Laurie would prick baby Lilly's heels—sometimes 10 to 15 times a day—to check her blood sugar levels and then give her food if her blood sugar levels were too low or give her insulin if her blood sugar levels were too high. “It made me very sad, as a mother, to have to withhold food from my baby when she was hungry because her blood sugar was [too]

high,” Laurie says.

Likewise, if Lilly's blood sugar was too low, Laurie would have to feed her, even

if Lilly wasn't hungry. Laurie kept tubes of cake frosting around for such times, because it was a ready source of sugar she could rub on Lilly's gums to help bring her out of her “lows.”

Maintaining Lilly's health those first few years meant constant vigilance. Laurie says that diabetes was like having an extra child in the family, in addition to Lilly and her brother, Nathan, and sister, Charlotte. “You can never let your guard down when you have a child with diabetes, let alone a baby with diabetes,” Laurie says.

When Lilly was 4 years old, she was put on an insulin pump after she had two seizures in 3 months due to low blood sugar. The pump made insulin administration easier by providing insulin through a tube that stays inserted under the skin. The pump helped stabilize Lilly's blood sugar

levels and also freed her from having to endure individual insulin shots throughout the day.

However, changing the pump's infusion set still required being stuck with a long needle, which Lilly hated. “When my mom was trying to stick the needle in me, I would run away from her,” Lilly remembers. “And it was very frustrating, obviously, for my mom, because that was

something I needed.” The pump made life easier, but Lilly was still too young to monitor her blood sugar on her own.

Living with diabetes as a young child can be confusing. When Lilly was put on the insulin pump, she says, “A lot of my friends would ask, ‘Oh, why do you have to wear that?’ And I really didn't know.”

She needed an adult to calculate how much insulin she required and operate the pump.

Living with diabetes as a young child can be confusing, Lilly says. She didn't always understand why she couldn't do the same things that her siblings did, such as go on sleepovers, or why her mother had to come along and monitor how much she ate at birthday parties. And sometimes other children didn't understand, either. When Lilly was put on the insulin pump, she says, “A lot of my friends would ask, ‘Oh, why do you have to wear that?’ And I really didn't know.”

A New Diagnosis and Hope for Easier Treatment

After Lilly's diagnosis, her parents became involved with the type 1 diabetes advocacy organization, JDRF. In June 2006, her father, Mike,

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attended a meeting sponsored by JDRF where a speaker mentioned new research on a rare form of diabetes called “monogenic” diabetes. Mike was immediately intrigued, as the description of monogenic diabetes fit Lilly perfectly.

Monogenic forms of diabetes result from changes in a single gene (as opposed to other forms of diabetes, which result from the activity of multiple genes). Monogenic diabetes accounts for about 1 to 5 percent of all cases of diabetes in young people. A subset of monogenic diabetes is called neonatal diabetes, which is diagnosed before 1 year of age and which is often misdiagnosed as type 1 diabetes. In most cases of monogenic diabetes, the person has an altered form of a gene involved in insulin production, and that change reduces the amount of insulin the person makes or secretes into the bloodstream. Most excitingly, as Mike heard at the meeting, a common and inexpensive class of oral medication used to treat type 2 diabetes, called sulfonylureas, had shown promise as a treatment in specific types of monogenic diabetes. These medications help the body’s beta cells release insulin. A “transition” therapy had been tested that involved slowly replacing insulin treatment with sulfonylurea pills.

Laurie was skeptical, but hopeful. After years of constant testing and insulin administration, the idea that Lilly could instead just take some pills seemed preposterous. Nonetheless, Mike and Laurie decided to have Lilly’s DNA tested. The test confirmed that Lilly had monogenic diabetes and that she was a candidate for the transition to sulfonylurea therapy. But this therapy had

not been widely used in the United States, and there were no guarantees that sulfonylurea would be able to replace Lilly’s insulin treatment. Finally, the transition attempt might also cause Lilly’s blood sugar levels to fluctuate wildly, a frightening proposition for a family that had spent so much time working to keep Lilly’s blood sugar in a healthy range.

Lilly, then 6 years old, also had reservations. When her parents explained what would be involved—a hospital stay, and then taking pills instead of needing her pump—she started crying. “I did *not* want to go to a hospital,” Lilly remembers. “I did *not* like them at that time, and I was not happy going.” Additionally, she had lived her whole life needing insulin and had grown very emotionally attached to her pump. “It was something I’d always had when I was younger...,” Lilly explains. “It was very important to me.”

Despite these reservations, the fact that Lilly’s health and quality of life could be greatly improved ultimately convinced Lilly and her family to take the risk and go ahead with the treatment. Lilly would be starting first grade that fall, and they hoped that she could start school no longer needing insulin.

Trading One Therapy for Another

To begin the therapy transition, Lilly’s clinical team cut her insulin dose in half and gave her a small dose of the oral sulfonylurea medication. It was a balancing act to find the right dosage of the new medication while reducing her insulin use.

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At first, the results were frightening: Lilly's blood sugars went, in Laurie's words, "sky high." Lilly was also scared, at first. Because she was a pioneering patient in this sort of procedure, there was a lot of attention focused on her, a lot of hospital staff coming and going, and a lot of tension all around. Lilly says that her friends were an incredible source of support while she was in the hospital. Their visits were a comfort and a welcome time of normalcy where Lilly could play with her friends.

"They helped me get through hard times," Lilly says.

When a blood test showed that the treatment was working, Laurie says, "We all started crying and hugging, and it was just this incredible moment."

On day 4 of the transition, Lilly reached a dramatic turning point. When she entered the hospital, a blood test had confirmed that her beta cells were not releasing insulin. By day 4, the medication had allowed her insulin dosages to be significantly reduced. Then, another test showed that her beta cells were now able to release insulin into her bloodstream. The new medication was working. When the doctor informed them of the good news, Laurie says, "We all started crying and hugging, and it was just this incredible moment. Even the doctor was blown away."

By the time Lilly left the hospital on day 5, she was still taking small doses of insulin, but her doctors were confident that the family could continue her treatment transition at home. On day 9—August 23, 2006—Lilly took off her insulin pump for the last time. Then, after taking her medication (but no insulin) for the night, Lilly celebrated with a big

bowl of ice cream while her parents looked on nervously. An hour later, her blood sugar levels were completely normal.

A Life Without Insulin Therapy

When asked how it felt to no longer need insulin therapy, Lilly says, "It felt really good, because I got to be more independent at that time."

Finally, she could do many of the things that

other children her age could do, unconstrained by her diabetes. As of publication, the

sulfonylurea therapy has continued to keep Lilly's blood sugar levels in a normal range without the need for insulin for almost 10 years.

Unfortunately, not all people with diabetes can be helped by sulfonylureas. Lilly remembers hoping that her cousins and friends who have type 1 diabetes would be able to switch from insulin to pills. She was sad when her parents explained that the therapy wouldn't work for kids with type 1 diabetes, as they have a different form of diabetes than she has. Even for some people with monogenic diabetes, sulfonylurea treatment may work partially or not at all, depending on the genetic change that causes their disease and on their particular circumstances.

Lilly and her family have continued to share their story to raise public awareness about monogenic diabetes and the sulfonylurea therapy that can help some people with the disease. Most children

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are not tested for monogenic diabetes, and many health providers are not aware that infants with diabetes may have monogenic diabetes rather than type 1 diabetes.

Lilly's family's advocacy led to the first neonatal diabetes registry in the United States. In 2009, Illinois passed "Lilly's Law," establishing a registry of Illinois children diagnosed with neonatal diabetes before their first birthday. This registry helps doctors connect children and their families with appropriate treatments and could also help scientists identify new genes that cause neonatal diabetes. Laurie and Mike have also produced a television documentary featuring their and other families' stories ("Journey to a Miracle: Freedom from Insulin"), which was released in early 2015.

Support for scientific research is critically important, Laurie says. "Research takes time. It takes decades. Research builds on research that builds on research." Thinking about the research that led to Lilly's diagnosis of monogenic diabetes from a DNA test, and her new treatment, Laurie explains, "This breakthrough seems sudden, but...it was decades in the making." Laurie is excited about the benefits that future research might bring.

Looking to the Future, Reflecting on the Past

Now 16 years old, Lilly's future is bright. A high school sophomore, she enjoys history and vocal ensemble. She attends theater and acting programs and has enjoyed performing for years. She particularly loves singing and dancing, and she wants to pursue a career in musical theater.

"Research takes time. It takes decades. Research builds on research that builds on research. This breakthrough [in Lilly's treatment] seems sudden, but...it was decades in the making."

And what of Lilly's old insulin pump, that symbol of her first 6 years on insulin? They still have it...in a closet. They sometimes bring out the pump at interviews, using it as a visual reminder of how their lives have changed. In the past, there was the pump and all the supplies that went with it...and now, there is only Lilly's pills.

"I feel like I am very lucky," Lilly says. When she thinks about being on insulin, and of all the other children and families dealing with diabetes, she is very thankful. "I just feel really grateful to have this amazing thing happen to me."

For more information on monogenic diabetes, please see www.niddk.nih.gov/health-information/health-topics/Diabetes/monogenic-forms-diabetes-neonatal-diabetes-mellitus-maturity-onset-diabetes-young/Pages/index.aspx

