



Mitochondria are small structures inside of cells that generate much of the “fuel” cells use to perform their functions. In this image, several mitochondria (yellow) attach to a fat droplet (red) in a liver cell. This attachment may facilitate transport of fat molecules into the mitochondria where they are metabolized into energy for the cell.

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Diabetes, Endocrinology, and Metabolic Diseases

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

Diabetes is a debilitating disease that affects an estimated 25.8 million people in the United States—or 8.3 percent of the total population—and is the seventh leading cause of death.¹ Diabetes lowers average life expectancy by up to 15 years,² increases risk of death from cardiovascular disease 2- to 4-fold, and is the leading cause of kidney failure, nontraumatic lower limb amputations, and, in working-age adults, blindness.¹ In addition to these human costs, the estimated total financial cost for diabetes in the United States in 2007—including costs of medical care, disability, and premature death—was \$174 billion.¹ Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.¹

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin; and type 2 diabetes, in which the body becomes resistant to insulin signaling, with subsequent impaired insulin production. 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 adults and the majority of children and youth with diagnosed diabetes.¹ It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing beta (β) cells of the pancreas. If left untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. Thus, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels to near the normal levels achieved by functional β cells. Thus, researchers are actively seeking new

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

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

methods to improve blood glucose monitoring and insulin delivery, as well as working to develop β cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.¹

Type 2 diabetes is associated with several factors, including older age and a family history of the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.³ Type 2 diabetes occurs at elevated rates among minority groups, including African Americans, Hispanic and Latino Americans, American Indians, and Native Hawaiians and Pacific Islanders.¹ Gestational diabetes is also a risk factor: women who have had gestational diabetes have a 35 to 60 percent chance of developing diabetes—mostly type 2 diabetes—in the next 10 to 20 years.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 79 million adults in the United States 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 high 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. Moreover, follow-up research has shown that this benefit of reduced diabetes risk can persist for at least 10 years.

Type 2 diabetes was previously called “adult-onset” diabetes because it is predominantly diagnosed in

older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. 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. Because diabetes often becomes more difficult to control over time, people diagnosed in their youth may find it even more challenging to control their blood glucose levels as they get older and thus prevent or delay the development of complications. In addition, increasing rates of type 2 diabetes in girls may lead to increasing numbers of 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 vicious 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. Exploring interrelationships between some of these diseases is an important and informative facet of this work—for example, diabetes is becoming an increasing problem for people with cystic fibrosis, as life expectancy for these individuals has improved due to advances in cystic fibrosis treatment. In parallel, based

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

on knowledge from past scientific research investments, the NIDDK is vigorously pursuing studies of prevention and treatment approaches for these diseases.

GENETICS OF TYPE 2 DIABETES

Uncovering Genetics Contributing to Diabetes

Health Disparities: Researchers scanning the genome have uncovered genetic factors that may account for at least part of the elevated risk for type 2 diabetes in African American and South Asian populations. Detailed maps of common genetic variation derived from the Human Genome and HapMap projects have led to a renaissance in the ability to study the genetics of human disease. For example, geneticists using the genome-wide association studies (GWAS) approach have found over 60 gene regions that affect risk for type 2 diabetes. However, while genetic factors are thought to contribute significantly to the higher burden of the disease in many non-white populations, the early studies focused on people of European descent. This was because the genome maps originally designed for GWAS analysis had not adequately captured genetic variation common in non-white populations to permit these studies. To better understand how genes affect diabetes health disparities, researchers have redesigned these tools and begun using them to perform GWAS with samples that are specific to populations with the highest burden of type 2 diabetes.

In one study, researchers used GWAS to look for genetic differences between African Americans who either had or did not have type 2 diabetes. Genetic markers that were more common in one group than in the other were then confirmed using samples from a larger number of African American study participants. This approach led to the discovery of one novel genomic location that appears clearly to influence the risk for type 2 diabetes in African Americans, as well as four other genomic locations that may be involved, although the data are less certain.

In a separate study, researchers examined DNA samples from South Asians, a population which also has elevated risk for type 2 diabetes relative

to people of European heritage. This approach led to the discovery of six genomic regions affecting type 2 diabetes risk in South Asian peoples. One of these regions includes *GRB14*, a gene encoding a protein that binds the insulin receptor and is thought to affect insulin sensitivity. Mice lacking *GRB14* are lean, and more sensitive to insulin than normal mice. Another region includes *HNF4A*, a gene in which previously discovered mutations were found to reduce pancreatic insulin production and cause a rare disease called maturity onset diabetes of the young. While neither study reported discovering the precise genetic differences within the identified genomic regions that promote type 2 diabetes in African Americans or South Asians, or that provide Caucasians with relative protection from the disease, further studies looking in the 11 genomic regions identified by the 2 studies could begin to explain the cause of type 2 diabetes health disparities.

Palmer ND, McDonough CW, Hicks PJ, et al. A genome-wide association search for type 2 diabetes genes in African Americans. PLoS One 7: e29202, 2012.

Kooner JS, Saleheen D, Sim X, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet 43: 984-989, 2011.

Understanding the Genetic Risk for

Type 2 Diabetes: Analyses of genetic variations associated with type 2 diabetes provide insight into how these variations affect an individual's risk for this disease. An explosion of technologies and tools has enabled researchers to conduct large studies to compare the genomes of thousands of people with and without type 2 diabetes to identify genetic variants that affect the likelihood of developing the disease. Whereas genetic risk for some diseases can be accounted for by a few variants with large effects, the current set of over 60 risk variants for type 2 diabetes only explains a small percentage of the genetic risk for the disease. In addition, how most of these variants alter the risk of type 2 diabetes is unknown. Toward the goal of elucidating the effects of these variants on disease risk, researchers are utilizing a number of

different approaches, taking advantage of the latest developments in tools and technologies, as evidenced by two recent studies.

In the first study, scientists hypothesized that identification of additional risk variants and the function of the genes they affect will illuminate a limited set of molecular pathways that influence development of type 2 diabetes. By conducting a meta-analysis in which they pooled data from over 34,000 people with type 2 diabetes and nearly 115,000 people without the disease, the researchers identified 10 previously unreported type 2 diabetes risk variants. Two of the newly discovered associated variants showed sex-differentiated associations; one was more significantly associated with type 2 diabetes in males, and the other in females. The researchers used several analytical approaches to identify pathways and networks that may be involved in the development of type 2 diabetes. The investigators found that some of the key processes influenced by type 2 diabetes risk genes include regulation of cell division, signaling by proteins secreted by fat cells, and regulation of gene activity (whether a gene is turned “on” or “off”) by a protein called CREBBP.

In another study, the researchers focused on assessing the extent to which the previously identified type 2 diabetes genetic risk variants affected the activity of genes that are either nearby or distant from them (often on a different chromosome). Genes are typically regulated in the sense that they can be kept inactive, when not needed, or utilized to varying degrees. The regulatory elements—which typically include DNA near the gene and the proteins that interact with that DNA—are thus analogous to the components of a dimmer switch: they control the extent to which a gene is activated (or “expressed”) in any given cell. The investigators sought to determine which of the known type 2 diabetes risk variants affect expression levels of nearby genes, which affect expression of distant genes, and which have little impact on gene expression. They found that, in general, genetic variants associated with type 2 diabetes affected genes at a distance from the genetic variation itself—either on a different chromosome than that containing the variant or on the same chromosome, but not nearby.

This result is surprising because many researchers assumed that the identified genetic variants altered the activity of a gene or genes in the same chromosomal neighborhood as the variant. Because specific genes are often more active in one tissue than in another, the investigators examined the impact of the type 2 diabetes risk genes on gene expression in multiple tissues involved in the disease. The data revealed many tissue-specific effects, which may eventually help uncover how specific genetic variants exert their effects on disease risk. By increasing understanding of the genetic factors that influence development of type 2 diabetes, researchers hope to use this knowledge to tailor prevention and treatment strategies to individuals and to develop new therapeutic approaches.

Morris AP, Voight BF, Teslovich TM, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 44: 981-990, 2012.

Elbein SC, Gamazon ER, Das SK, Rasouli N, Kern PA, and Cox NJ. Genetic risk factors for type 2 diabetes: a trans-regulatory genetic architecture? Am J Hum Genet 91: 466-477, 2012.

EXAMINING COST-EFFECTIVENESS OF APPROACHES TO PREVENT TYPE 2 DIABETES

Interventions To Prevent Type 2 Diabetes Provide Good Return on Investment: New research has shown that using either of two interventions to prevent or delay type 2 diabetes in people at high risk for the disease would be a very cost effective way to improve their health and quality of life. The landmark Diabetes Prevention Program (DPP) clinical study demonstrated that an intensive lifestyle intervention designed to achieve modest weight loss through a combination of diet and exercise lowered type 2 diabetes rates by 58 percent, and that the generic diabetes medication metformin reduced diabetes rates by 31 percent, relative to placebo. Subsequently, researchers with the follow-up DPP Outcomes Study (DPPOS) showed that the health benefits of both DPP interventions persisted

for at least 10 years. In the new report, DPP researchers examined per person costs of the interventions during the trial and follow up, total direct medical costs outside the DPP/DPPOS, and measures of quality of life over 10 years. They found that the lifestyle intervention was cost-effective—that is, its modest net cost was well justified by the benefits of diabetes prevention, overall improvements in health, and the reduction in other health care costs. The new research also found that, although health benefits from metformin treatment were more limited than those conferred by the lifestyle intervention, the use of this inexpensive drug in the DPP population yielded a modest cost savings. The greater reduction in health care costs from the more expensive lifestyle intervention was nearly enough to offset the cost of the intervention, so that the lifestyle intervention was highly cost effective and nearly cost neutral. Previous research showed that metformin was most effective among DPP participants who were less than 60 years of age when the trial began, and among those with a history of gestational diabetes.

Throughout the study, quality of life as measured by mobility, level of pain, emotional outlook, and other indicators was consistently best in the lifestyle group, compared to metformin or placebo. These findings are particularly encouraging in light of other NIDDK-supported research demonstrating the feasibility of substantially reducing the cost of the lifestyle intervention by delivering it to groups in community-based settings such as YMCAs. It is hoped that this approach will yield both health care savings and better health for many people, if widely implemented.

Diabetes Prevention Program Research Group. The 10-year cost-effectiveness of lifestyle intervention or metformin for diabetes prevention: an intent-to-treat analysis of the DPP/DPPOS. Diabetes Care 35: 723-730, 2012.

TREATING TYPE 2 DIABETES IN YOUTH

Trial Highlights Challenge in Treating Youth with Type 2 Diabetes: Results reveal that type 2 diabetes is more difficult to treat in youth with the disease than in adults. Although type 2 diabetes is most commonly

diagnosed in people over the age of 40, an increase in childhood obesity and other factors has led to a significant rise in cases in people under 20 years of age. Since development of long-term complications in adults is related to both duration of diabetes and control of blood glucose levels, the increasing prevalence of people diagnosed with type 2 diabetes during childhood is a major public health concern. Prior to this study, it was unknown whether treatments developed for adults would work well for younger patients. Metformin, the first-line drug of choice among adults with type 2 diabetes, is currently the only oral medication approved for use in children.

The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) trial tested how well three treatment approaches controlled blood glucose levels in youth ages 10 to 17. At the start of the study, the 699 participants had type 2 diabetes for less than 2 years and were either overweight or obese (as measured by a body mass index at the 85th percentile or greater). Participants were randomly assigned to receive either metformin alone; metformin and another diabetes drug, rosiglitazone, together; or metformin plus intensive lifestyle changes aimed at helping participants lose weight and increase physical activity. Unfortunately, metformin alone failed to maintain acceptable, long-term blood glucose control in 51.7 percent of youth over an average follow-up of 46 months—a much higher failure rate than expected. Metformin plus lifestyle changes failed 46.6 percent of the time, an improvement over metformin alone that is not statistically significant. The combination of metformin plus rosiglitazone was significantly better, but still failed 38.6 percent of the time over the follow-up period. Importantly, after the study began, the U.S. Food and Drug Administration restricted use of rosiglitazone because of studies linking the medicine to a higher risk of heart attacks and stroke in adults. Thus, at this point, rosiglitazone is not recommended for use in children.

These results suggest treatment with metformin alone may be inadequate for a majority of youth with type 2 diabetes, and that type 2 diabetes is a more aggressive disease in youth than in adults. The findings also emphasize the importance of preventing and treating

childhood obesity, so that overweight and obese youth do not develop type 2 diabetes. Most of the medications widely used by adults to control type 2 diabetes have not been studied in children. Additional research is needed to determine whether any of these, singly or in combination, can safely and reliably control blood glucose in young people with the disease.

Zeitler P, Hirst K, Pyle L, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med 366: 2247-2256, 2012.

MONITORING CHILDREN AT RISK FOR TYPE 1 DIABETES

Research on Preventing a Life-threatening Condition Associated with Type 1 Diabetes Onset: Researchers have shown that young children being monitored from birth in a long-term research study have a reduced risk of developing diabetic ketoacidosis (DKA) at onset of type 1 diabetes compared to children in the general population. DKA is caused by profound insulin deficiency. When symptoms of diabetes are not recognized, the disease can progress to life-threatening DKA. Scientists estimate that as many as 40 percent of children who develop type 1 diabetes under age 3 and 60 percent of children under age 2 have DKA at disease onset. This condition could lead to coma or death, underscoring the need to find ways to prevent it. Toward that goal, researchers examined whether children participating in The Environmental Determinants of Diabetes in the Young (TEDDY) study had reduced rates of DKA. TEDDY is following over 8,000 newborns at high genetic risk of developing type 1 diabetes until they are 15 years old to identify environmental triggers of disease. Researchers hypothesized that children in TEDDY may have lower rates of DKA at disease onset because they are being monitored frequently and the parents know that their child is at genetic risk of developing type 1 diabetes, both of which could lead to earlier diagnosis and prevention of DKA.

To determine whether participants in TEDDY have lower rates of DKA, researchers compared children who were diagnosed with the disease in TEDDY with

children who were diagnosed in the general population of countries where TEDDY is being conducted (United States, Germany, Finland, Sweden), using data from studies and national registries. In children diagnosed under age two, the percent with DKA was significantly lower in TEDDY than in the general population. In children diagnosed under age five, the percent with DKA was lower in TEDDY compared to the general populations in the United States and Germany, but not different in Finland and Sweden, where the disease is more common. The research suggests that DKA occurs less frequently in TEDDY children. The scientists note that it is currently cost-prohibitive to do the type of screening and monitoring being done in TEDDY on a population-wide scale. However, the research sheds light on approaches that could be used in the future to achieve the goal of preventing this life-threatening condition.

Elding Larsson H, Vehik K, Bell R, et al. Reduced prevalence of diabetic ketoacidosis at diagnosis of type 1 diabetes in young children participating in longitudinal follow-up. Diabetes Care 34: 2347-2352, 2011.

CONTROLLING BLOOD GLUCOSE CAN PREVENT LOSS OF KIDNEY FUNCTION

Intensive Blood Glucose Control Reduces Kidney Disease: New results show that controlling blood glucose early in the course of type 1 diabetes yields huge dividends, preserving kidney function for decades. The landmark Diabetes Control and Complications Trial (DCCT) began in 1983, but because it can take years for early signs of diabetes complications to develop, it was not until 1993 that sufficient time had passed for the trial to prove that intensive blood glucose control reduced early signs of kidney dysfunction and other complications in people with type 1 diabetes. However, because more serious impairment of kidney function or kidney disease can take even longer to develop, researchers could not determine the effect of intensive therapy on the development of kidney disease at that time. DCCT participants were invited to join the DCCT follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, and

today, nearly 3 decades after the start of the DCCT, about 95 percent of DCCT participants continue to be followed to determine the long-term effects of the therapies beyond the initial treatment period. Now, after an average 22-year follow-up, EDIC researchers reported that controlling blood glucose can prevent loss of kidney function and is likely to reduce kidney failure. Compared to conventional therapy, near-normal control of blood glucose—beginning soon after diagnosis of type 1 diabetes and continuing an average 6.5 years—reduced the long-term risk of developing kidney disease by 50 percent. This finding—along with previous DCCT/EDIC research demonstrating the benefit of intensive blood glucose control in reducing the risk of eye, nerve, and cardiovascular complications—reinforces the importance of early, intensive blood glucose control in people with type 1 diabetes. DCCT and EDIC illustrate the value of long-term studies, have revolutionized disease management, and led to greatly improved outcomes for people with type 1 diabetes.

de Boer IH, Sun W, Cleary PA, et. al. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 365: 2366-2376, 2011.

NEW APPROACHES TO PREVENT AUTOIMMUNITY

Small Molecules Hold Promise To Prevent Type 1 Diabetes: New research has identified promising small molecules to prevent development of type 1 diabetes. Because type 1 diabetes results from inappropriate activity of the immune system, scientists are pursuing potential treatments that suppress this harmful activity. However, many agents that suppress the harmful aspects also suppress the protective aspects of the immune system and, therefore, can have toxic side effects and increase a person's risk for infection. Scientists are investigating therapies to selectively suppress the specific cells involved in autoimmune diseases like type 1 diabetes. Small molecules have proven valuable for affecting the function of genes, cells, and biological pathways, and hold great promise for the prevention of type 1 diabetes. A key challenge, however, is to identify small molecules that can

selectively modulate a specific biological process or disease state. Two recent reports utilized different strategies to find promising new molecules for the prevention of type 1 diabetes.

In previous work, a group of scientists developed a small molecule that selectively affected Th17 cells—immune system cells that produce a protein called IL-17 and have been previously implicated in autoimmune diseases. They demonstrated that the molecule affected Th17 cells by binding to and repressing two proteins, called ROR α and ROR γ , whose activity is required for the development of Th17 cells. In new research, these scientists synthesized a modified version of their molecule that selectively binds to and suppresses ROR γ , but not ROR α . This was done because other research suggested that suppressing ROR γ alone inhibits development of Th17 cells, and it is preferable to develop small molecules that specifically target the disease process, to reduce the chance of adverse side effects. This new molecule still inhibits IL-17 production, suggesting that the molecule is a potent repressor of ROR γ activity and has potential for the treatment of autoimmune disease.

Another group of scientists, searching for a potential drug to prevent type 1 diabetes, screened a large library of small molecules and identified one that alters a key autoimmune reaction in this disease. With knowledge of the structure of a protein that confers type 1 diabetes risk (a form of the MHC class II protein), the researchers used computer simulation to identify small molecules that are likely to bind this protein, and then tested these molecules further in experiments with cells and mice. They hypothesized that these small molecules, by binding the protein, could alter how the protein presents insulin to immune cells called T cells, which is important in the development of autoimmunity. Indeed, further analyses demonstrated that certain of these small molecules could enhance or inhibit the T cell response to insulin; enhancing certain types of T cell responses could potentially prevent or dampen autoimmunity. One small molecule they identified, called glyphosine, enhanced a T cell response to insulin in mouse cells, increased production of an anti-inflammatory protein (called IL-10),

and, when given to mice genetically susceptible to type 1 diabetes, prevented development of the disease. Importantly, giving glyphosine to human cells from people with type 1 diabetes suggested that it may enhance a protective type of T cell response in humans as well. Further research with these promising small molecules will determine whether they have utility in humans to prevent type 1 diabetes.

Kumar N, Lyda B, Chang MR, et al. Identification of SR2211: a potent synthetic ROR γ -selective modulator. ACS Chem Biol 7: 672-677, 2012.

Michels AW, Ostrov DA, Zhang L, et al. Structure-based selection of small molecules to alter allele-specific MHC class II antigen presentation. J Immunol 187: 5921-5930, 2011.

Homing in on Mechanisms To Promote Immune Tolerance—Implications for Combating

Autoimmune Diseases: Researchers discovered new details of a molecular mechanism that is critical to promoting immune tolerance of the body's own tissues, organs, and cells. In autoimmune diseases, like type 1 diabetes, the immune system launches a misguided attack against substances and tissues normally found in the body. In a person without autoimmune disease, immune cells are normally generated that recognize the body ("self") in addition to those that recognize foreign entities such as pathogenic bacteria or virus. In a process called "tolerance," these self-recognizing cells are muted or destroyed. Loss of tolerance, therefore, plays a role in the development of autoimmune diseases, and understanding how tolerance works and what goes wrong is critical to prevent and treat autoimmune diseases.

Previous research shed light on this process with the discovery that a protein, called AIRE, is critical in promoting tolerance. AIRE, by turning genes on and off, generates a wide variety of "self" proteins in the thymus. The presence of these "self" proteins in the thymus promotes tolerance. However, AIRE does not generate all the "self" proteins that are present in the body, so researchers hypothesized that there must be AIRE-independent mechanisms at work. To examine other potential mechanisms, scientists focused on

understanding the role that a specific type of immune cell—a dendritic cell—plays in promoting tolerance. Dendritic cells can be found in immune organs like the thymus, and at interfaces between the body and the environment, like skin or the surfaces of the airway and intestine. These cells constantly "sample" the environment; they take up proteins and particulates and then "present" the sample to cells that determine whether or not to launch an immune attack.

The researchers studied one type of dendritic cell, a plasmacytoid dendritic cell (pDC). They examined this cell type because they previously found that it contained a protein called CCR9, which is involved in homing of other immune cells to the thymus. Therefore, they speculated that CCR9 may also be involved in homing pDCs to the thymus. To test this hypothesis, they genetically engineered mice to lack the CCR9 gene and found that these mice had fewer thymic pDCs compared to normal mice, suggesting that CCR9 was important for pDC recruitment to the thymus. In addition, they intravenously injected mice with pDCs without CCR9 and observed that only a small percentage made it to the thymus. The researchers next wanted to determine whether pDCs had a role in transporting "self" proteins to the thymus. They loaded pDCs with an experimental "self" protein and injected those cells into genetically modified mice. The injected pDCs homed to the thymus and, importantly, led to the destruction of immune cells that recognized the "self" protein. Further experiments showed that pDCs within mice were able to "pick up" an experimentally introduced particulate and transport it to the thymus, a possible therapeutic approach for inducing tolerance.

These results suggest that pDCs contribute to tolerance through CCR9-dependent transport of "self" proteins to the thymus, a process that complements AIRE-regulated mechanisms. Further research will determine whether a similar mechanism occurs in humans and whether disruption of this process is associated with type 1 diabetes and/or other autoimmune diseases. This finding could also present an exciting opportunity to identify novel approaches to promote tolerance in autoimmune diseases.

Hadeiba H, Lahl K, Edalati A, et al. Plasmacytoid dendritic cells transport peripheral antigens to the thymus to promote central tolerance. *Immunity* 36: 438-450, 2012.

BETA CELLS AND DIABETES

Beta Cell Markers of Maturity: Researchers have discovered new markers that allow them to distinguish between mature β cells that produce insulin in a regulated fashion and immature β cells that do not. A major goal of diabetes research is to turn stem and/or progenitor cells in the laboratory into insulin-producing β cells that can be transplanted into people. Importantly, those cells must release insulin properly in response to glucose to be a viable therapeutic option. Significant progress has been made in generating immature β cells in the lab, but these cells do not respond appropriately to glucose. In order to develop strategies to turn immature β cells into mature cells, it would be useful if scientists had experimental markers that helped them distinguish between the two cell types. The authors discovered that immature cells isolated from newborn mice released insulin in response to lower glucose levels than mature cells isolated from adult mice. In other words, the immature cells had a lower “glucose threshold” for releasing insulin. The researchers then compared how the levels of various proteins differed between the two groups of cells. They found that a protein called urocortin 3 was present at much higher levels in functionally mature β cells than in immature cells in both mice and humans. These discoveries provide important new tools to scientists testing experimental strategies to generate mature β cells in the lab—they could use differences in glucose threshold and urocortin 3 levels as markers of β cell maturation. These findings can facilitate research to turn stem and/or progenitor cells into mature β cells as a possible therapy for people with diabetes.

Blum B, Hrvatin SS, Schuetz C, Bonal C, Rezanian A, and Melton DA. Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat Biotechnol* 30: 261-264, 2012.

Signaling Pathway Found To Regulate Regeneration of Insulin-producing β Cells: Two recent studies identified a signaling pathway that regulates β cell regeneration and could be targeted for new therapies. β cells, which produce insulin, are destroyed by the immune system in people with type 1 diabetes and may not function normally in people with type 2 diabetes. Identifying ways to replace the β cells and restore insulin-producing capacity could benefit people with type 1 or type 2 diabetes, and is a major goal of research. One approach to replace β cells is through regeneration, such as by coaxing existing β cells, which reside in the pancreas, to proliferate and generate new β cells. In particular, small molecules that could promote β cell regeneration could be therapeutically useful, particularly if they had limited off-target effects and could be taken orally. Toward the goal of identifying such small molecules, two different research groups participating in the Beta Cell Biology Consortium conducted screens to identify small molecules that enhanced β cell regeneration.

In one study, researchers screened over 7,000 small molecule compounds in a zebrafish model in which β cells were partially destroyed through genetic manipulation. They identified five compounds that doubled the number of β cells in the animals. Interestingly, four of the five compounds targeted the same cellular pathway—the adenosine signaling pathway. Further experiments focused on the most potent compound, called NECA. NECA was found to promote β cell proliferation specifically, without significantly affecting proliferation of other pancreatic cell types (*e.g.*, glucagon-producing cells) or cells in other tissues (*e.g.*, liver, gut). To determine whether NECA had a similar effect in mammals, the researchers studied an adult mouse model of diabetes in which β cells were depleted. After 15 days, β cell mass was 8-fold larger in the NECA-treated mice compared to control mice, suggesting that NECA promotes β cell regeneration in the mouse model as well.

In a separate study, researchers developed a high-throughput small molecule screening system using rat islets, and used that experimental platform to screen about 850 compounds for their ability to

increase β cell proliferation. Two compounds increased β cell proliferation 2- to 3-fold above control levels; the compounds also increased β cell proliferation in mouse and pig islets. Both of the compounds are inhibitors of adenosine kinase, an enzyme in the adenosine signaling pathway. They tested one of the compounds in mice, and it increased β cell proliferation but did not affect proliferation of other cell types tested, suggesting that inhibiting adenosine kinase may selectively promote β cell proliferation.

Both studies point to the adenosine signaling pathway as a key regulator of β cell regeneration. The preliminary results with the compounds tested in these studies are promising because they may promote β cell proliferation selectively, which would be critically important in any therapy used in people. The research suggests that therapies targeting this pathway could potentially be used to promote β cell regeneration to treat diabetes.

Andersson O, Adams BA, Yoo D, et al. Adenosine signaling promotes regeneration of pancreatic β cells in vivo. Cell Metab 15: 885-894, 2012.

Annes JP, Ryu JH, Lam K, et al. Adenosine kinase inhibition selectively promotes rodent and porcine islet β -cell replication. Proc Natl Acad Sci USA 109: 3915-3920, 2012.

Small Molecule Enhances β Cell Function:

Researchers have discovered a family of small molecules that increases insulin production in pancreatic β cells, and thus could be explored for potential use in diabetes therapy. They built on previous research showing that small molecules called isoxazoles (Isx) increase levels of the NeuroD1 protein in nerve cells and promote neuronal cell development. NeuroD1 is also a key regulator of β cell development and maturation, as well as insulin production in β cells. Therefore, the scientists tested whether Isx had an effect on β cells, which are found in clusters called islets in the pancreas. They examined human islets that had been in laboratory culture for a long period of time (2-12 months), during which they lose their ability to secrete insulin in response to glucose. They discovered that treating these human islets

with Isx increased the levels of cellular factors that regulate insulin production, β cell function, and β cell development, resulting in enhanced insulin production. Experiments using mouse pancreatic cells in culture showed that treatment with Isx stimulated insulin secretion in response to glucose. These findings make Isx one of only a few known molecules that improves β cell function dramatically, although further research is needed before Isx or related molecules could be tested in people. Because impaired β cell function is central to both type 1 and type 2 diabetes, this research could potentially lead to new therapeutic approaches for both forms of the disease.

Dioum EM, Osborne JK, Goetsch S, Russell J, Schneider JW, and Cobb MH. A small molecule differentiation inducer increases insulin production by pancreatic β cells. Proc Natl Acad Sci USA 108: 20713-20718, 2011.

RESEARCH ON DIABETIC NERVE PAIN

Newly Discovered Pathway for Nerve Pain

in Diabetes: A factor produced during glucose metabolism may be responsible for painful peripheral neuropathy in diabetes. Many people with diabetes suffer from peripheral neuropathy, nerve damage that starts in the feet and can cause either pain or loss of feeling in the toes and then feet, legs, and hands. While scientists have suspected that elevated blood glucose levels play a role in painful peripheral neuropathy, the exact mechanism(s) has been unclear. In a recent study, researchers found that, compared to people without diabetes, people with type 2 diabetes have higher circulating levels of methylglyoxal, a small molecule that is produced during glucose metabolism. Intriguingly, people with diabetes and foot pain had significantly higher levels of this molecule than either people without diabetes or people with diabetes but no pain. Methylglyoxal is metabolized by a cellular enzyme called glyoxylase 1, or GLO1. Peripheral nerves, such as those detecting sensations in the hands and feet, have low GLO1 activity; because there may be insufficient GLO1 to metabolize excess methylglyoxal, these nerves may be particularly vulnerable to accumulating high levels

of the molecule. To determine whether methylglyoxal exacerbates pain responses, the researchers used a variety of approaches to experimentally raise the levels of this molecule in mice, including chemically inducing diabetes in some and inhibiting the GLO1 enzyme activity in others. They found that, compared to untreated animals, these mice became hypersensitive to both heat and mechanical stimuli. In contrast, the scientists were able to “rescue” diabetic mice from hypersensitivity to heat either by increasing GLO1 enzyme levels in peripheral nerves, or by injecting the mice with a molecule that gets rid of excess amounts of methylglyoxal. Through additional experiments, the researchers uncovered evidence for a molecular mechanism that could explain how exposure to excess methylglyoxal increases the excitability of pain-signaling peripheral nerve cells and enhances activation of brain regions involved in pain processing.

At this time, few effective treatments exist for painful diabetic neuropathy. The novel discovery of a metabolically driven mechanism that increases sensitivity to potentially painful stimuli not only opens up a new line of study, but could potentially lead to new therapeutic approaches for this pain condition in people with diabetes.

*Bierhaus A, Fleming T, Stoyanov S, et al. Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nat Med* 18: 926-933, 2012.*

METABOLIC REGULATORS OF HEALTH AND DISEASE

Switching Fuel Sources from Fat to Sugar—Novel Role for Mitochondrial Enzyme and Implications for Type 2 Diabetes: Researchers have discovered an important role for an enzyme called carnitine acetyltransferase (CrAT) in regulating fuel selection under different nutritional conditions. CrAT is located in mitochondria, the structures in cells that extract energy from a variety of fuel sources. Glucose is their primary fuel, but mitochondria can switch to burning fat when glucose levels are low, such as during a

fast. Previous research showed that the mitochondria of people with type 2 diabetes are not as adept as those of healthy people when it comes time to switch back to using glucose following a meal—and also suggested that the reason may relate to a problem with CrAT. The major function of CrAT is to catalyze the transfer of the “acetyl” portion of a molecule called acetyl-CoA to an essential nutrient called carnitine. Unlike CoA, carnitine can take acetyl groups out of the mitochondria. This is important, because acetyl-CoA is a major by-product of mitochondrial energy production, and, if it accumulates, it can interfere with the work of other mitochondrial enzymes, including those involved in glucose metabolism. The new research explores the role of CrAT in metabolism, and helps explain why fuel switching is reduced in people with type 2 diabetes.

The researchers genetically engineered mice to lack CrAT in their muscles, finding that such animals became insulin resistant and had high blood glucose levels, two conditions associated with prediabetes and diabetes. These results suggested that CrAT was playing a role in regulating metabolism. Experiments on mitochondria isolated from the animals’ muscles showed that they burn more fat than do normal mitochondria, but were impaired in their ability to switch to burning glucose. The researchers found that experimentally reducing CrAT activity in laboratory cultures of human muscle cells had similar effects to those observed in mice without CrAT, reducing capacity to switch from using fats to using glucose for cellular fuel. Because people with type 2 diabetes have a defect in fuel switching, the researchers hypothesized that people with the disease may have reduced levels of CrAT in their muscles. Indeed, they found that muscles of people with type 2 diabetes seem to make less CrAT than those of people without the disease. Other experiments suggested that loss of CrAT led to the expected build-up of acetyl-CoA, apparently inhibiting enzymes that are important in glucose metabolism and thus preventing fuel switching to glucose. These observations suggested that it might be possible to help cells of people with type 2 diabetes get rid of the excess acetyl-CoA by providing supplementary carnitine, the nutrient to which CrAT transfers the acetyl group,

and thus making it easier for the enzyme to do its job. The researchers tested this approach in a pilot study in older people with modestly elevated blood glucose levels who were given carnitine supplements for 6 months. Insulin sensitivity improved, and the activity of a cellular enzyme involved in glucose metabolism increased, suggesting that carnitine supplementation may help promote fuel switching to glucose. These results confirm that CrAT is an important regulator in transitioning between glucose and fat metabolism. The preliminary results in the human pilot study, if confirmed through placebo-controlled trials, suggest that carnitine supplementation or other strategies to target CrAT may prove to be viable therapeutic approaches to treatment or prevention of type 2 diabetes.

Muoio DM, Noland RC, Kovalik JP, et al. Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. Cell Metab 15: 764-777, 2012.

Solving a Decades-old Mystery Yields Insight into

Metabolic Disease: After decades of investigation, a transport complex that carries the metabolite pyruvate from the cytoplasm into the mitochondria has been identified. Pyruvate is a key intermediate in carbohydrate, fat, and amino acid metabolism. It is usually produced in the cytoplasm, and then is transported into the mitochondria—the “powerhouses” of the cell—where it is metabolized further. How pyruvate gains entrance into the mitochondria has remained a mystery. Solving this mystery has important implications because altered metabolism underlies common diseases, such as type 2 diabetes and obesity, and, as shown in this study, a less common but devastating disease caused by impaired mitochondrial pyruvate metabolism.

Using an elegant series of genetics, metabolomics, and other analyses, scientists have identified two related proteins, mitochondrial pyruvate carrier 1 (MPC1) and mitochondrial pyruvate carrier 2 (MPC2), that form a complex that transports pyruvate from the cytoplasm into the mitochondria. The genes *Mpc1* and *Mpc2* were initially identified as part of an ongoing effort to characterize mitochondrial proteins that are conserved

through evolution. Both yeast and fly mutants lacking the *Mpc1* gene showed reduced pyruvate metabolism; under certain conditions this resulted in poor growth or lethality. Experiments in yeast suggested that the defect was due to diminished entry of pyruvate into mitochondria, thus implicating MPC1 as a pyruvate carrier. Further evidence of MPC1’s role in pyruvate transport was provided by studying a chemical, UK-5099, that has been used experimentally to block the activity of pyruvate transport into mitochondria since 1975—even though scientists had not known the exact target of its effects. When assessed in this study, UK-5099 was shown to be a potent inhibitor of MPC1-mediated pyruvate transport in yeast. Further studies in yeast designed to evaluate the interaction between MPC1 and MPC2 suggest that these proteins form a multimeric complex (more than one copy of each protein present) in the mitochondrial membrane. Building on the findings in yeast and flies, the researchers next examined whether MPC1 and MPC2 are involved in transport of pyruvate in mammals. In mouse cells in laboratory culture, turning off the *Mpc1* or *Mpc2* gene impaired pyruvate metabolism. In addition, genetics studies in humans showed that in three unrelated families, children with a devastating disease caused by impaired mitochondrial pyruvate metabolism had mutations in *Mpc1*. Thus, the findings suggest that MPC1 and MPC2 are also serving as pyruvate carriers in humans, and give insight into a serious genetic disease.

The findings of this study solve a mystery that has perplexed scientists working in this area for many years. Moving forward, the identification of these pyruvate carrier proteins opens up new research directions that could be pursued for treating a variety of metabolic diseases.

Bricker DK, Taylor EB, Schell JC, et al. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. Science 337: 96-100, 2012.

Fat Cell Gene May Protect Against

Type 2 Diabetes: New research describes how adipose (fat) tissue can protect the body from type 2 diabetes by influencing systemic insulin sensitivity. Insulin, a hormone produced in the

pancreas, stimulates the uptake of glucose in multiple tissues, providing fuel for cells. Adipocytes (fat cells) are sensitive to fluctuations in nutrient availability; they are able to sense and respond to changes in glucose levels. For example, in response to insulin and high levels of glucose, glucose uptake is stimulated in adipose tissue by the transporter protein GLUT4. In obesity, however, GLUT4's activity is reduced in adipose tissue, blocking glucose from entering the fat cells and leading to adverse whole-body metabolic effects, including insulin resistance. How this altered adipose tissue glucose metabolism causes whole-body insulin resistance has remained a key question.

Knowing that GLUT4 is central to regulation of adipose tissue metabolism, the researchers analyzed changes in the levels of gene activity in mice that were genetically engineered to have high levels of GLUT4 in adipose tissue or to lack GLUT4 specifically in that tissue. This approach enabled the scientists to identify factors involved in the adipocyte glucose response. They observed that a set of genes involved in producing fats was upregulated in mice with high levels of adipose GLUT4 and downregulated in mice lacking adipose GLUT4. High levels of these fat-producing genes in fat cells were previously shown to be associated with the metabolic benefits of enhanced glucose tolerance and insulin sensitivity. Mice with high levels of adipose GLUT4 displayed both of these characteristics, despite being obese. The scientists discovered that, in the mice, the enhanced glucose tolerance and insulin sensitivity required a factor called ChREBP.

To extend their findings to humans, the researchers looked at levels of expression—gene activity—of the *ChREBP* and *GLUT4* genes and insulin sensitivity in over a hundred individuals without diabetes (with normal glucose levels) and with widely ranging body mass index values. They found that adipose levels of *ChREBP* expression correlated strongly with *GLUT4* expression levels and insulin sensitivity, consistent with a role for ChREBP in GLUT4-mediated glucose metabolism. Because not all obese people are insulin resistant, the investigators looked at *ChREBP* levels and insulin-stimulated glucose uptake in obese individuals with widely ranging insulin sensitivity.

They observed that *ChREBP* was strongly correlated with insulin sensitivity, suggesting that ChREBP could be a protective factor against obesity-associated insulin resistance. Additional experiments to understand the mechanism of this protection identified a novel form of ChREBP, called ChREBP- β , and higher levels of this form specifically predicted insulin sensitivity in humans.

These results indicate the importance of ChREBP in regulating adipocyte and whole-body glucose control and insulin sensitivity, showing that some features of fat cells can play a critical role in protecting the body against type 2 diabetes. Selective activation of ChREBP- β in adipocytes could be a new therapeutic strategy for preventing and treating type 2 diabetes and obesity-related metabolic diseases.

Herman MA, Peroni OD, Villoria J, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 484: 333-338, 2012.

Suppression of Fat Tissue Inflammation Promotes Insulin Sensitivity: Scientists have uncovered a key factor in the link between obesity and type 2 diabetes. Insulin resistance is a condition in which the body produces the hormone insulin but does not use it properly. Because this condition leads to increased risk for type 2 diabetes and heart disease, understanding how insulin resistance develops is critical toward efforts to prevent or reverse it. During excess weight gain, a type of immune cell migrates into and accumulates in adipose (fat) tissue and promotes chronic, low-grade inflammation, which contributes to the development of insulin resistance. Another type of immune cell, the regulatory T cell (Treg), is abundant in the adipose tissue of lean, but not overweight, mice and humans. The presence of Tregs in adipose tissue helps to protect mice from developing insulin resistance. However, the molecular mechanisms that regulate the Treg cell population in adipose tissue remained undefined.

In a recent study, researchers found that a protein known to be essential for fat cell development is also critical for maintaining adipose tissue Treg cell numbers in mice.

The protein, called PPAR- γ , resides within adipose tissue Tregs, as well as within fat cells themselves. PPAR- γ controls the activities of different sets of genes, depending on the particular cell type. The scientists genetically engineered mice to lack PPAR- γ exclusively in Tregs, and observed a significant reduction in Treg cell numbers within adipose tissue. Pioglitazone, an insulin-sensitizing drug used to treat type 2 diabetes, is known to activate PPAR- γ . The researchers thus sought to understand whether PPAR- γ in Tregs was responsible for pioglitazone's insulin-sensitizing effects. To test this idea, the scientists treated obese mice with pioglitazone, and observed an increase in Treg cells in adipose tissue, suggesting that the drug treatment can influence the abundance of adipose tissue Treg cells. In these mice, pioglitazone treatment improved metabolic traits such as insulin resistance, glucose tolerance, and insulin tolerance. However, in mice that lacked PPAR- γ in Treg cells, pioglitazone did not increase the abundance of adipose tissue Tregs and improved metabolic traits were not observed. These findings reveal a new role for PPAR- γ in suppressing adipose tissue inflammation by recruiting Tregs to adipose tissue. This study also defines a new molecular pathway for pioglitazone action—one that might be exploited to develop new and effective therapeutics for type 2 diabetes.

*Cipolletta D, Feuerer M, Li A, et al. PPAR- γ is a major driver of the accumulation and phenotype of adipose tissue T_{reg} cells. *Nature* 486: 549-553, 2012.*

Molecular Insights Could Lead to New Drugs To Extend Healthy Lifespan: Researchers have identified a molecular mechanism that could spur the development of new drugs that may lengthen healthy lifespan. A few years ago, the drug rapamycin was found to extend the lifespan of mice. However, rapamycin treatment—though U.S. Food and Drug Administration-approved for use in preventing rejection of transplanted organs and other applications—also comes with significant side effects, including increased susceptibility to infection (because it suppresses the immune system), toxicity to certain organs, and—surprisingly—elevation of blood glucose levels, especially after meals. Rapamycin inhibits a protein called mTOR, which integrates

signals from nutrients and growth factors. In new research, scientists sought to understand the molecular mechanisms by which rapamycin extends lifespan yet elevates blood glucose levels. They found that in mice, rapamycin reduced the activity of two different mTOR-containing protein complexes, designated mTORC1 and mTORC2. Using genetically engineered mice to tease out the effects of the different mTOR complexes, they found that the mechanisms by which rapamycin is affecting lifespan and glucose levels are independent: glucose elevation resulted from the mTORC2 inhibition, while the lifespan extension was an effect of inhibiting mTORC1. These findings may pave the way toward the development of new therapies to extend healthy lifespan by targeting mTORC1, while avoiding some of the unwanted side effects associated with rapamycin.

*Lamming DW, Ye L, Katajisto P, et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* 335: 1638-1643, 2012.*

TREATING HYPOTHYROIDISM

Alternate Therapy May Benefit Some People with Hypothyroidism: New research has found that some patients who continue to experience symptoms of hypothyroidism despite receiving conventional therapy may benefit from administration of a synthetic form of the thyroid hormone triiodothyronine (T_3). Hypothyroidism is a disorder that occurs when the thyroid gland does not make enough T_3 to meet the body's needs. T_3 regulates metabolism—the way the body uses energy—and affects nearly every organ in the body. Without enough of the hormone, many of the body's functions slow down. The disorder—which can stem from a variety of causes—affects about 4.6 percent of the U.S. population age 12 and older. Symptoms of hypothyroidism include fatigue, weight gain, facial “puffiness,” intolerance of cold, and many other problems. The thyroid secretes T_3 along with thyroxine, also known as T_4 , a less potent form of thyroid hormone, which is converted to T_3 by various tissues throughout the body. Because T_4 is more stable than T_3 , and the body can generate T_3

from T_4 , current standard therapy for hypothyroidism is a once-daily pill containing a synthetic form of T_4 .

However, experiments have shown that not all tissues in hypothyroid mice dosed with T_4 achieve as high a level of T_3 as they need, which may explain why a subset of people with the disorder continue to experience some of its symptoms even when receiving what should be an adequate dose of T_4 . Previous studies have tested T_3/T_4 combination therapy, but the results of these studies have been inconsistent. The present study compared therapy with T_4 alone to therapy with T_3 alone in 14 participants who had continued to experience some symptoms of hypothyroidism while receiving standard therapy. The study was designed so that after 6 weeks those receiving T_3 were switched to T_4 , and vice versa—with neither the patient volunteers nor the researchers knowing until after the trial was over which participants received T_3 in the first half of the trial, and which received it second. Participants took the medicines orally, three times per day, before meals. The researchers found that participants lost weight on T_3 relative to T_4 , showed significant improvement in their levels of cholesterol and other blood fats, and experienced no serious side effects. A longer, larger trial will be necessary to determine the long-term safety and efficacy of this approach. However, this preliminary study suggests that while T_4 remains an excellent approach for most people with hypothyroidism, particularly given the relative convenience of its once-daily dosing, thrice-daily dosing of T_3 might be a good alternative for those who continue to experience symptoms.

Celi FS, Zemskova M, Linderman JD, et al. Metabolic effects of liothyronine therapy in hypothyroidism: a randomized, double-blind, crossover trial of liothyronine versus levothyroxine. J Clin Endocrinol Metab 96: 3466-3474, 2011.

CYSTIC FIBROSIS RESEARCH

Research Identifies Key Hurdle in Quest for Cystic Fibrosis Treatment: Two recent studies have provided a key insight on what has been a puzzling roadblock in attempts to develop a new therapy for the majority

of people with cystic fibrosis (CF). CF is an inherited disease of the glands that make mucus and sweat, with serious consequences for the lungs, pancreas, liver, intestines, sinuses, and sex organs. Thanks to the discovery of new antibiotics and improved symptom treatment, the average life expectancy for a CF patient has nearly quadrupled from about 10 years in the 1960s to about 37 years today. Indeed, some people who have CF are living into their 40s, 50s, or older. Despite these gains, life expectancy for CF patients remains much lower compared to healthy adults. CF treatment regimens can be arduous, and managing the disease can be a great challenge for patients and their families.

The development and 2012 approval of a new drug, ivacaftor, has therefore been a tremendous boon to the roughly 5 percent of people with CF who have at least 1 copy of a mutation designated *G551D* in *CFTR*, the cystic fibrosis gene. Ivacaftor was developed through a search for compounds that help stabilize the *G551D* version of the CFTR protein, allowing the protein to reach the cell surface and do its job. In patients with *G551D*, the effect of ivacaftor is to substantially alleviate many of the most serious CF symptoms. Unfortunately, researchers have not yet been successful in finding a compound that can provide a similar benefit to patients with the most common CF mutation, designated *ΔF508*. (Although many CFTR mutations have been identified, about 90 percent of people with CF have at least 1 copy of *ΔF508*.) Two recent studies identify the likely reason why an ivacaftor-like approach—identification of compounds that help stabilize the *ΔF508* form of the CFTR protein—has yet to benefit patients with this mutation. CFTR is a large protein with several “domains,” *i.e.*, sections of the protein that “fold” into specific three-dimensional structures with distinct functions. The *ΔF508* mutation changes a part of the CFTR protein called the first nucleotide binding domain (NBD1), eliminating a single amino acid that was previously known to be essential for proper NBD1 folding and function. The new findings show the missing amino acid also plays a key role in interaction of NBD1 with an adjacent CFTR domain, designated the 4th intracellular loop (ICL4). Using different methods, the two groups of researchers reached the same conclusion: that proper

CFTR folding and function require restoration not only of proper NBD1 folding, but also stabilization of the NBD1-ICL4 interaction. Drug discovery screens to date have focused on improved NBD1 folding and function, and have not focused on improving the interaction with ICL4. Now the search is on for a drug or drug combination which corrects both of the $\Delta F508$ structural issues. If that search is successful, the resulting treatment may one day restore significant CFTR function to people with this CF mutation, potentially reducing the burden of the disease for most people with CF, and allowing them to lead longer, healthier lives.

*Rabeh WM, Bossard F, Xu H, et al. Correction of both NBD1 energetics and domain interface is required to restore $\Delta F508$ CFTR folding and function. *Cell* 148: 150-163, 2012.*

*Mendoza JL, Schmidt A, Li Q, et al. Requirements for efficient correction of $\Delta F508$ CFTR revealed by analyses of evolved sequences. *Cell* 148: 164-174, 2012.*

Researchers Ferret Out a New Model for Cystic Fibrosis-related Diabetes: The recently developed ferret model of cystic fibrosis (CF) is providing key insights into the development of an important consequence of the disease in humans—CF-related diabetes (CFRD), which is associated with worsening lung function, dangerous weight loss, and increased mortality. Improved antibiotics and other therapeutics have dramatically increased life expectancy for people with CF, roughly quadrupling it over the last 3 decades. As this has happened, complications like CFRD—which affects almost half of CF patients over the age of 30—are of increasing concern to people with CF and those helping them manage their disease. Although the physiological reasons why CF often leads to CFRD are unknown, researchers have found it to be quite different from other major forms of diabetes. Unlike type 1 diabetes, there is no autoimmune attack on the insulin-producing β cells of the pancreas in CFRD. Insulin resistance—a central feature of type 2 diabetes—is often observed to some degree in CFRD, but while

obesity is a risk factor for type 2 diabetes, underweight is a much more likely concern in CFRD.

Mouse and pig models of CF have helped clarify what goes wrong in some of the many organ systems affected by the disease. But the utility of these models has been limited by differences between the animals and humans in the way CF affects certain organs and tissues. Of particular note, CF results in enormous damage to the pancreases of pigs even before birth, while CF mice rarely (if ever) develop CFRD. In contrast, CF ferrets have pancreatic problems that more closely mimic human CF, so researchers used that animal model to help uncover the underlying causes of CFRD. They discovered that CF ferrets had early and gradually worsening regulation of glucose levels, not unlike what is often observed in people who have CF. The researchers found that the CF ferrets have smaller clusters (“islets”) of β cells at birth, and fewer islets at death, than do control animals without CF. Intriguingly, they also discovered that laboratory cultures of islets from newborn CF ferrets did not respond appropriately to changes in glucose levels: compared to islets from ferrets without the disease, the CF ferret’s β cells secreted less insulin at high glucose levels, and also secreted more insulin at low glucose levels. The net effect observed in living CF ferrets was relatively even insulin levels that do not fluctuate normally in response to rising or falling blood glucose levels. These observations show that even at birth, insulin secretion by the pancreas is abnormal in animals with CF. Taken together, the results suggest that despite the insulin resistance often observed in CFRD, the disease quite likely stems from defects in β cell function, and that while some of these β cell problems are present from birth, they are exacerbated by progressive damage to the pancreas. Further study of the ferret model of CF may help researchers find improved ways to prevent or treat CFRD in people with CF.

*Olivier AK, Yi Y, Sun X, et al. Abnormal endocrine pancreas function at birth in cystic fibrosis ferrets. *J Clin Invest* 122: 3755-3768, 2012.*

A TIME TO SLEEP AND A TIME TO METABOLIZE

New discoveries about the relationships between metabolism and circadian rhythms provide possible new approaches to averting conditions such as obesity and diabetes.

Why does a rapid change in time zone or seasonally setting clocks forward or back throw people for such a loop? We can chalk this up to internal timepieces. In animals and humans, biological “circadian clocks” regulate behaviors and bodily processes—including sleep/wake cycles, changes in blood pressure, and body temperature fluctuations—to harmonize these activities with daily, rhythmic changes in the environment, most notably day/night cycles. One commonly observed sign of the strong influence of circadian rhythms is jet lag, the sleep disturbances and other symptoms that occur after flying across multiple time zones. However, disrupting circadian rhythms can have more insidious consequences.

In humans, misaligning normal circadian rhythms with behaviors such as sleep and eating—for example, by working the night shift—increases vulnerability to diabetes, obesity, and other metabolic problems. One likely reason for these problems is the fact that the circadian clock has a critical relationship with metabolic pathways important to maintaining normal energy balance. For example, the synthesis of glucose (sugar) and fats and the release of glucose into the blood by the liver are governed by the circadian clock. Understanding how circadian rhythm and metabolism are linked therefore could help in the design of strategies to reduce vulnerability to metabolic diseases, and is an area of intense investigation.

Researchers working on the link between the circadian clock and metabolism are actually faced with an additional layer of complexity. Animals and humans possess two types of clocks—a “core” circadian clock, located in the brain, and “peripheral,” tissue-specific circadian clocks. The core or master clock responds to cues such as light and dark, nutrient uptake, and

temperature. For this clock, a set of core genes has been identified. Some of these clock genes encode activator proteins that help “turn on” target genes, and some of them encode repressor proteins that help “turn off” target genes. Cleverly, the clock genes interact to generate cyclical oscillations in the levels of proteins they encode—*e.g.*, products of activator genes “turn on” repressor genes, and products of repressor genes “turn off” activator genes, so that levels of activator and repressor proteins wax and wane. This leads to downstream effects that occur with regularity within each 24-hour period. The master clock also synchronizes the tissue-specific clocks, which use much of the same genetic machinery. However, the clocks present in each cell can also act and respond on their own, setting up local, tissue-specific rhythms governing gene activation or repression, and subsequent cellular processes—including metabolic activities.

Intriguingly, researchers have found that some of the factors regulating tissue-specific clocks are shared with pathways regulating metabolism. But is there a single factor (or factors) that acts as a key molecular link between circadian rhythms and metabolism? The Rev-erb- α protein has emerged as a candidate. Rev-erb- α is a nuclear receptor protein, meaning that one part of the protein functions as a sensor for a specific signal molecule, or ligand, while another part of the protein functions to bind to DNA in the cell’s nucleus and regulate gene activation. The ligand for Rev-erb- α is heme, a small molecule that is integral to many metabolic pathways and whose cellular levels oscillate in a circadian manner. When bound by heme, Rev-erb- α binds to specific target DNA sequences to repress activation of nearby genes. In the context of the circadian clock, Rev-erb- α is believed to regulate how much protein is made by a master clock gene called *Bmal1*. In the context of metabolism, Rev-erb- α regulates genes involved in glucose metabolism, regulates lipid and bile acid production in the liver, and is necessary for the maturation of fat cells from precursor cells. But, because mice genetically

engineered to lack Rev-erb- α do not display a strongly disrupted circadian rhythm, a question has remained as to whether Rev-erb- α plays an accessory role in the circadian clock or is truly a central factor that could link the clock with metabolism.

A recent report has helped to clarify the role of Rev-erb- α . Through a series of experiments in mice, researchers found evidence suggesting Rev-erb- α doesn't just regulate the *Bmal1* gene, but acts cooperatively with the BMAL1 protein at numerous DNA target sites in the liver to regulate the activity of both metabolic and circadian clock genes. Moreover, they found that there is overlap in activity between Rev-erb- α and the closely related protein Rev-erb- β , which could explain why lacking only Rev-erb- α doesn't result in disturbed circadian rhythms. To test this idea further, the researchers used a wheel-running behavior test, a standard method to ascertain circadian dysfunction in mice. In this test, mice first have their rest and active periods artificially synchronized to a cycle of 12 hours of light alternating with 12 hours of darkness. During this initial step, baseline measures of wheel-running rhythms are established; unlike humans, mice are nocturnal and will normally show the most activity during the dark. Then, the mice are put in constant darkness, and their wheel-running behavior is assessed for changes. In the absence of the alternating light/dark cues, normal mice will maintain a circadian rhythm of rest and activity, although the total cycle length will shrink to slightly less than 24 hours. When shifted to constant darkness, mice lacking only one Rev-erb protein showed little or no change in wheel-running behavior compared to normal mice, although mice lacking Rev-erb- α experienced a further shortening of the cycle length. In contrast, mice lacking both the Rev-erbs throughout their bodies showed weak synchronization during the light/dark cycle and, when put into total darkness, decreased and severely fragmented activity and other features indicating circadian dysfunction. As these features are also found in mice lacking BMAL1, their findings suggest both that the Rev-erbs can compensate for each other and that there is a much more central role for Rev-erbs in rhythmic behaviors.

The increasing evidence that Rev-erbs play a central role in both circadian rhythm and metabolism makes them promising therapeutic targets. Knowing that the natural ligand for Rev-erbs is heme, scientists recently synthesized and tested two small molecules for their ability to stimulate Rev-erb effects on circadian rhythms and metabolic outputs in mice. When administered to mice, these molecules were able to repress Rev-erb-responsive metabolic genes in the liver and the oscillation of a core circadian clock gene in the brain. Interestingly, when subjected to the wheel-running behavior test, mice that received single injections of either of the two drugs during the total darkness phase experienced transient but drastic disruptions in running behavior. However, the drugs were much less disruptive when tested under normal light/dark conditions, resulting in only a delay in activity. Drug administration to normal weight mice caused a loss in fat weight and an increase in metabolic rate without any change in food intake and a decrease in activity levels. This appeared to be due to an increase in the levels of enzymes that burn fat. When administered to mice with diet-induced obesity, the drugs improved their metabolic profile, with much greater weight loss compared to normal weight mice, a drop in triglycerides, and cholesterol levels cut nearly in half. The results in both normal weight and obese mice suggest that the drugs exert their effects through Rev-erbs by modifying genetic programs in a way that leads to increased burning of fatty acids and glucose, improving the metabolic profile.

Another potential therapeutic target that has emerged from the study of circadian rhythm and metabolism is a protein called HDAC3. HDAC3 is an enzyme that causes transitory structural changes along the chromosomes called "histone modifications," which affect gene activation. When HDAC3 is recruited to sites in the genome, genes at those sites tend to be turned "off," and when it is absent, those genes are free to be turned "on." Scientists have studied the activity of HDAC3 in the liver and found that, directed by Rev-erb- α , HDAC3 drives circadian oscillations in the activation of genes controlling fat synthesis in liver cells. Now, a team of researchers has made

another intriguing discovery. They found that while depleting HDAC3 in adult mouse livers leads to potentially harmful accumulation of fat in the liver, the fat is sequestered within little droplets surrounded by protective coatings and the mice actually have better insulin sensitivity than mice with normal levels of HDAC3, without any changes in body weight. By examining molecular pathways in the liver involved in the generation, storage, and burning of fat and in the generation of glucose, the researchers determined a likely mechanism: it appears that the constant, rather than rhythmic, activation of lipid-synthesizing and sequestering genes caused by the absence of HDAC3 redirects precursor molecules away from making glucose and toward the synthesis and storage of fat. These findings uncover a previously unknown means for regulating glucose generation and insulin sensitivity in the liver. By distinguishing liver fat accumulation caused by disruption of circadian control of metabolism from liver fat accumulation caused by diet, the findings also suggest that there are multiple pathways by which the liver can end up storing fat, and their potential for harm—or relative benefit—will need to be considered during therapeutic development and application.

In addition to identifying key molecular aspects of circadian control of metabolism, there is another facet to this problem: can we use the knowledge that there is circadian control of metabolism to develop behavioral strategies as well as pharmacologic ones to thwart metabolic disease? Researchers recently tested in mice whether timing of feeding, even with a high-fat diet, can influence weight gain and related metabolic problems. They compared four groups of mice: one group was given a normal diet and allowed to eat at any time (“*ad lib*”); another group was given a normal diet, but only during an 8-hour window at night, the natural feeding time for mice (“time-restricted”); a third group was given a high-fat diet *ad lib*; and the fourth group was given a high-fat diet, but time-restricted. At the end of 100 days, the researchers found that, while the mice in all four groups consumed the same number of calories,

mice on the time-restricted feeding regimens appeared to have better metabolic profiles. Strikingly, despite having the same diet, mice on the high-fat diet but time-restricted feeding regimen were leaner than their *ad lib* counterparts. At the molecular level, it appears that, by imposing a feeding rhythm, the time-restricted feeding regimen “reprogrammed” activation of pathways governing glucose and fat metabolism in the liver and prevented the circadian and metabolic dysfunction that can occur with a high-fat diet.

As the understanding of the role of factors such as the Rev-erbs and HDAC3 in the circadian control of metabolism continues to evolve, it is helping researchers to test new and existing therapeutic compounds that may help in the fight against diseases such as diabetes and obesity. At the same time, the improved glucose tolerance, protection from obesity, and protection from fatty liver disease seen in mice on a time-restricted, high-fat diet—as well as the metabolic improvements seen in mice on a time-restricted, normal diet—is encouraging, and provides hope that behavioral strategies based on understanding the relationship between circadian rhythms and metabolism can also be developed to prevent metabolic disease.

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New Indo-U.S. Collaboration in Diabetes Research



On June 12, 2012, U.S. Department of Health and Human Services Secretary Kathleen Sebelius (center) and India's Honorable Ghulam Nabi Azad, Minister of Health and Family Welfare (second from right), signed a joint statement on collaboration on diabetes research. They were witnessed by Dr. Griffin P. Rodgers, Director of the NIDDK (left), Dr. V.M. Katoch, Secretary of India's Department of Health Research and Director-General, Indian Council of Medical Research (second from left), and the Honorable Krishna Tirath, India's Minister of State for Women and Child Development (right). The signing took place at the Hubert H. Humphrey Building in Washington, DC. *Photo credit: Chris Smith, HHS*

Diabetes is a global scourge, affecting tens of millions of people around the world. In a move to address this international health challenge, on June 12, 2012, Health and Human Services Secretary Kathleen Sebelius and India's Health and Family Welfare Minister Ghulam Nabi Azad signed a joint statement to begin a formal research relationship in diabetes between the National Institutes of Health and the Indian Council of Medical Research (ICMR). Through this new collaboration, the two nations hope to accelerate efforts to better understand the mechanisms underlying diabetes and to identify innovative solutions to prevent and treat the disease.

About 26 million Americans have diabetes¹; in India, the burden is estimated at over 62 million people.² Millions more in both countries are at increased risk for developing diabetes and its health complications. The challenge of diabetes for the United States and India is complex and multi-faceted: in the United States, persons from racial and ethnic minorities, and those of

lower socio-economic status, are disproportionately affected by diabetes. Across India, many challenges exist in accessing affordable health care, including diabetes care. Simultaneously, rapid economic growth and workforce transitions over the last few decades have led to changes in the Indian population's physical activity and diet, which further contribute to diabetes risk. In both nations, diabetes is increasingly striking in younger age groups, with potentially devastating implications for the health, well-being, and productivity of future generations.

In addition to sharing this burgeoning public health problem, both countries already conduct substantial research on diabetes, such as examining lifestyle interventions and metformin to prevent type 2 diabetes. The new joint statement provides greater opportunities for researchers in India and the United States to join forces in projects ranging from research to identify genes for diabetes to bettering public health efforts to manage

and treat diabetes. For example, one potential area of collaboration may be in studying why people of South Asian origin develop diabetes at a lower body mass index and waist circumference than people of other ethnic origins—a question of interest to both India and the United States, with its large South Asian population.

“Both the United States and India have a vested interest in improving our understanding of, and treatment for, diabetes and in finding economical ways to do both,” says NIDDK Director Dr. Griffin Rodgers, which will lead the U.S. role in the collaboration. “Initiating this research relationship will enable both countries to share expertise and engage each other in research to lessen the burden of diabetes—in the United States, India, and around the world.”

As a first step in partnering, the NIDDK and ICMR held a scientific workshop on February 4-6, 2013, in New

Delhi, India. The theme of this initial workshop was the development of affordable and practical approaches and technologies for preventing and managing diabetes and its complications. Both countries could benefit from such approaches and technologies, which are needed to reduce the human toll of diabetes and the high costs of care. The workshop convened diabetes researchers from India and the United States and asked them to identify scientific opportunities in diabetes prevention and management that could be pursued through collaborative efforts. NIDDK and ICMR plan to use these ideas in developing the next steps of the joint Indo-U.S. diabetes research initiative in 2013.

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

² Anjana RM, et al. *Diabetologia* 54 :3022-7, 2012. Epub 2011 Sep 30.

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A Dramatic Improvement in Care for Some People with Cystic Fibrosis

A recent breakthrough means that for the first time, some people with cystic fibrosis (CF) can lead their lives with greatly reduced symptoms of the disease. CF is an inherited disease affecting numerous organs throughout the body, including the lungs, pancreas, and intestines. Thick mucus in the lungs of people with CF promotes infections by *Pseudomonas aeruginosa* bacteria, which thrive in the mucus and gradually damage lung tissue. Digestive and pancreatic manifestations of the disease lead to delayed growth and malnutrition. While research has led to enormous strides in CF treatment during the last few decades, dramatically increasing life expectancy for those with the disease, treatments can be arduous and time-consuming, and those with CF remain highly susceptible to dangerous infections and other serious complications. The breakthrough—development of a new medication that can overcome the fundamental molecular flaw in people with a particular mutation of the CF gene—stems from decades of research.

A Big Advance from Sweating the Small Stuff: The Genetic Cause of CF

The new treatment has its roots in some of the earliest characterization of the disease. One characteristic of CF that may at first glance seem strangely unconnected to its profound lung, pancreatic, and other consequences is that people with the disease have particularly salty perspiration. Indeed, for many years CF was typically (and quite accurately) diagnosed with a sweat test. Salt is a key ingredient in perspiration, enabling sweat glands to release water in response to increases in body temperature. Evaporation of the water helps cool the body, but the

salt—after it has done its job of helping move water out of sweat glands—is no longer needed on the skin. In fact, because the body needs salt (for many purposes in addition to producing more sweat), sweat glands normally reabsorb a portion of what they have released. The first clue to the function of the CF gene came in 1983, with the discovery that the salty skin of people with CF is caused by a defect in this reabsorption process. Specifically, the scientists found that sweat glands from CF patients are much less capable of absorbing chloride—one of the chemical components of salt—than are normal sweat glands. This finding suggested that the genetic mutation underlying CF results in the inactivation of a chloride transport protein.

But more dramatic progress was not possible until the gene encoding that chloride transporter was discovered. Through painstaking genetic analysis, NIDDK-funded investigator Dr. Lap-Chee Tsui and colleagues had identified a region of human chromosome 7 as the likely location of the CF gene. Using the standard gene discovery methods of the era, it might have taken decades to find the CF gene within this region, but Tsui recruited Dr. Francis Collins and his group to join in the effort. Dr. Collins—now the director of the NIH—had recently published a method for making jumps across difficult-to-analyze sections of DNA. This “chromosome jumping” approach dramatically hastened the search. Although it was still a mammoth undertaking, the two groups of investigators announced in 1989 that working together they had succeeded in identifying the gene that is mutated in CF. Confirmation might have been simpler if DNA samples from some people with CF had a large deletion, chromosome rearrangement, or at least

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a mutation that would radically change or eliminate the gene, but that did not turn out to be the case. Instead, combing through the genetic information of the gene, they found that just over two-thirds of the chromosome 7s they tested from people with CF (each of whom has two chromosome 7s, one from each parent) were missing the code for just one of the protein's predicted 1,480 amino acid building blocks. Subtle though this tiny deletion seemed to be, they found that most people with CF had two copies of the gene with the deletion, and most of their parents had one copy with the deletion and one without it. Significantly, none of the parents had two copies with the deletion. This was very strong evidence that the researchers did indeed have the correct CF gene, and that the single amino acid deletion was the most common disease-causing mutation.

This signal discovery made it possible to deduce some basic characteristics of the encoded protein, including tell-tale motifs that suggest it functions in cell membranes, and binds a molecule called ATP, which is key for many cellular processes. The arrangement of these motifs was similar to those of a previously characterized protein involved in the transport of substances out of cells. Putting this together with the observations about salty sweat, the researchers correctly guessed that they were characterizing a protein involved in the transport of salt. The gene's discoverers dubbed it the "cystic fibrosis transmembrane conductance regulator" (CFTR). We now know that when the protein binds ATP, it opens a pore on the cell surface through which negatively charged chloride ions (one of the two chemical components of table salt) can travel. The positively charged sodium ions of salt then follow in other ways, keeping the electrical charge balanced in cells. Importantly, this ion flow has the key effect of drawing water along via osmosis, to balance salt concentrations. The flow of water out of lung cells,

made possible by movement of chloride ions through healthy CFTR proteins, hydrates the thin, protective layer of mucus on their surface. In people with CF, neither salt nor water flow—so what should be thin, protective mucus becomes thick, sticky, and an ideal habitat for lung-damaging bacteria.

Progress from Studying CFTR Mutations

The mutation the researchers discovered that causes elimination of a single amino acid was dubbed $\Delta F508$ (because it deletes the 508th amino acid in CFTR, a phenylalanine, designated F), and was later shown to represent about two-thirds of CFTR mutations worldwide. Thus, about 90 percent of people with CF have at least one copy of $\Delta F508$. (Among people of European descent, about 1 person in 30 has a single copy of $\Delta F508$, along with a normal working copy of the CFTR gene, which is enough to avoid CF. Some evidence suggests that CFTR mutations are as common as they are because people with one mutated and one normal version were historically less likely to succumb to diseases like typhoid fever, tuberculosis, or cholera.) Although $\Delta F508$ is by far the most common mutation, about half of people with CF have at least one copy of one of the thousands of other known CFTR mutations. By studying these various CF-causing mutations, researchers have discovered a great deal about the CFTR protein, its function, the disease physiology of CF, and—ultimately—medicinal approaches to address those problems at the molecular level. For example, they found that $\Delta F508$ results in a protein that is unstable, and that is degraded before it gets to the cell membrane—which helped clarify the maturation process by which the cell prepares the protein for its role on the cell surface. Other mutations, such as one designated $G551D$, result in CFTR proteins that reach the cell surface in adequate quantities, but which fail to open and therefore do not allow the flow of chloride through the CFTR "gate." CF

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researchers therefore designated mutations like *G551D* that yield stable but non-functional CFTR proteins to be “gating” mutations. “Conductance” mutations gate normally, but contain a defective pore through which chloride cannot travel. The different types of mutations provide key information about the way the parts of CFTR work together to regulate the flow of chloride.

Intriguingly, researchers found that $\Delta F508$ -CFTR proteins could actually reach the cell surface—and even transport chloride—if made by cells grown in cool conditions (well below body temperature) in the laboratory. This suggested the tantalizing possibility that if they could somehow identify medicinal compounds that stabilize $\Delta F508$ at body temperature, or that allow CFTR proteins made with gating or conductance mutations to allow chloride flow, they might effectively be able to treat the fundamental molecular problem in CF.

Progress from Technological Innovation

CF researchers therefore sought to identify candidate medicines that could promote CFTR function. A key step in that discovery process was the creation of stable cell lines bearing various human CFTR mutations that could be grown in the laboratory. In principle, investigators could then expose the cells to different candidate drugs, and ask which allowed the transport of chloride. Checking for ion transport, however, is no easy task if it has to be performed one chemical at a time for hundreds of thousands of potential medicines. The work was therefore greatly advanced by the development of fluorescent markers of CFTR activity. For example, one NIDDK-supported group created a protein that fluoresces when exposed to ultraviolet light, but dims substantially when bound to ions like chloride. Another group employed chloride-sensitive-dyes. These innovations allowed the medicine hunters to create large arrays

of test chambers containing CFTR-mutant cells, accompanied by a different candidate drug in each chamber, and then use fluorescence to detect ion flow in many chambers at once. Screening hundreds of thousands of compounds, in this fashion, the searchers were able to identify a few that had properties they were looking for—helping different mutant CFTR proteins function better. The promising candidates they discovered were then chemically tweaked in various ways, to try to improve on their ability to promote chloride flow. Of course, these compounds might work beautifully in the laboratory, but to be useful as CF drugs, the compounds would have to be proven safe, and capable of reaching cells where they are needed. Thus, laboratory animals such as mice that have been engineered to have the same CFTR mutations found in humans were another critical resource for preclinical testing.

Among the compounds found to have the most promising qualities was one designated VX-770 by the pharmaceutical company where it was identified. Following animal testing and a preliminary dosage trial in humans, it was eventually shown in trials of increasing size and length to significantly improve function of the lungs, pancreas, and other affected organs in people with at least one copy of the *G551D* gating mutation. Based on these strong safety and efficacy results, the U.S. Food and Drug Administration approved VX-770 (as “ivacaftor,” marketed as “Kalydeco™”) in January, 2012. This is great news for people with CF in the United States who have at least one copy of the *G551D* mutation. It accounts for about 2 percent of CF-causing mutations in the United States, (meaning about 4 percent of U.S. residents with CF have a copy of the mutation), but is somewhat more common in Ireland, Scotland, Brittany (in France), and the Czech Republic. Several other, rarer gating mutations are also known, and it is hoped the drug

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may also prove valuable in patients with some of these forms of CFTR as well.

Although initial characterization of VX-770 and of a few other compounds identified through similar screening approaches suggested they may be of benefit to anyone with the much more common $\Delta F508$ mutation, this has unfortunately not proved to be the case. A drug does not have to restore complete function to CFTR to be of substantial benefit to people with CF—achieving 10 percent or more of chloride transport activity is expected to have a real impact on symptoms. So why have such efforts so far been unsuccessful in the case of $\Delta F508$? Two recent papers are helping provide an explanation: $\Delta F508$ not only interferes with folding of the first ATP binding domain of CFTR, it also disrupts interaction of this domain with a neighboring part of the protein. Because there are two physiological problems with the same protein, it is not enough to correct one of them. In fact, it may be necessary to improve the protein folding, the domain interaction, or both, by quite a bit more than 10 percent to achieve a net restoration of 10 percent of normal channel function. This is a substantially higher hurdle to cross, but the search may be facilitated by this better understanding of what such a drug (or combination of drugs) must achieve. Thus,

it may be productive to search for a compound that promotes a significant increase in chloride transport in cells with the $\Delta F508$ mutation in the presence of VX-770 or another compound, which by itself confers only a modest improvement.

Other drugs are in development for other types of CFTR mutations. For example, the CFTR gene can be thought of as an instruction list for the cell, indicating the order and identity of each of the CFTR protein's 1,480 amino acids. Some mutations change the list, so that the code for a particular amino acid is changed to a code instructing the cell's protein production machinery to stop adding amino acids to that protein. Drugs that can induce the cell to "read through" such "stop" instructions are also in development. Importantly, these ongoing CF drug searches are made possible by the same fundamental advances that enabled the development of VX-770 for people with CF and the $G551D$ mutation: discovery of the CFTR gene, characterization of the protein it encodes, analysis of the CF mutations, and the creation of CF cell lines, animal models, and fluorescent ion sensors. Thus, the quest continues for medicines to help more people with CF lead lives that are longer, healthier, and less burdened by the disease.

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Emerging Strategies To Combat β Cell Failure in Diabetes

Dr. Domenico Accili

Dr. Domenico Accili is a Professor of Medicine at Columbia University and Director of the Columbia University Diabetes Research Center in New York City. He is a graduate of the University of Rome and trained in internal medicine at the University Hospital Gemelli in Rome. Following a Fogarty Fellowship in the Diabetes Branch of the NIDDK's Intramural Research Program, he became Chief of the Section on Genetics and Hormone Action at the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Since 1999, he has served on the faculty at Columbia University's College of Physicians and Surgeons and as an attending physician at Columbia-Presbyterian Hospital.

Dr. Accili has served on several editorial boards; is a member of numerous advisory panels for academia, government, and industry; and his research has been published in leading medical journals. He has received numerous awards, including the 2003 Lilly Award for Outstanding Scientific Achievement by the American Diabetes Association, and is an elected member of the Association of American Physicians and the American Society for Clinical Investigation. His work is supported by the NIH, American Diabetes Association, Russ Berrie Foundation, and Brehm Coalition. Dr. Accili presented research findings from work conducted in his laboratory at the May 2012 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.

Loss of β Cell Function Drives Type 2 Diabetes

Glucose (a type of sugar) is the fuel of cells, providing energy that enables cells to carry out their various functions. Glucose can be derived from the consumption of food or from production by the liver. The essential hormone insulin, which is produced by the pancreas, stimulates the uptake of glucose from the blood by cells in muscle, fat, and liver, ensuring that these tissues are provided with the necessary fuel. β cells, which are found in the pancreas within cell clusters called islets, are the body's sole source of insulin. β cells carry out key functions in producing insulin and secreting insulin into the blood.

Diabetes is characterized by the body's failure to produce and/or respond appropriately to insulin, and results in the inability of the body to absorb and use glucose. In people with type 2 diabetes, cells do not properly react to insulin; this is referred to as "insulin resistance." As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the 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. Therefore, type 2 diabetes is characterized as a combination of two abnormalities: impaired insulin action and β cell dysfunction. It has been unclear, however, whether one of these drives the development of the disease more than the other, and whether doctors should focus treatment on one abnormality (and, if so, which one) or both.

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Scientists in the NIDDK's Intramural Research Program in Phoenix, AZ conducted a study to determine the sequence with which these abnormalities develop and their relative contributions to loss of maintenance of normal glucose tolerance (*i.e.*, progression to type 2 diabetes). By following a population at high risk of developing type 2 diabetes, the scientists observed that some people were able to maintain normal glucose tolerance, despite deterioration of the response to insulin action, because their insulin production increased. The increased amount of insulin was sufficient to shuttle the excess glucose from the bloodstream. The disease developed, however, in people whose β cells were unable to sustain increased insulin production: loss of β cell function was a key difference between those who progressed to the disease and those who did not. Therefore, impaired β cell function is a driver in the pathogenesis of type 2 diabetes. Understanding and combating β cell failure is thus critical to treating diabetes. Unfortunately, much is still unknown about how and why β cells fail, and treatments to improve β cell function effectively are lacking.

What Causes β Cell Failure?

Visualization of the progression to diabetes in rodent models has enabled scientists to document what happens to the pancreatic islet in great detail. Using experimental procedures to tag insulin with a dye visible by microscopy, they were able to mark β cells that are producing insulin and thus have not failed. Early in the course of the disease, when glucose levels are still normal and cells are developing resistance to insulin, scientists observed that, in the rodent model, the mass of β cells expands in response to insulin resistance. This is followed, as the disease progresses, by a decrease in β cell mass and loss of insulin. Dr. Accili and his colleagues hypothesize that human islets undergo a similar process in the development

of diabetes, but this has not yet been demonstrated. It has been suggested that the decrease in β cell mass results from the death of β cells, and there are numerous theories as to the cause(s) of β cell death. Dr. Accili noted that these proposed causes likely occur in β cells, but speculated that none of them is specific to the β cells of a person with diabetes, suggesting that none of the proposed causes is likely to be the driver of β cell failure in diabetes.

FOXO: An Important Sensor in Mammalian Metabolism

Dr. Accili's efforts to elucidate the driver of β cell failure has focused on a family of proteins—called FOXO—that regulates the levels of genes involved in a diversity of cellular processes, including the response to DNA damage, cell death, cell proliferation, stress tolerance, and longevity. Interestingly, FOXO proteins have also been implicated in the cell's response to insulin and metabolism. The link between FOXO proteins and insulin came to light in research from another laboratory studying the roundworm *C. elegans*. Scientists observed that modifying worms so that they lack the gene for the insulin receptor, a protein that is required for the function of insulin, led to accumulation of lipids (fat) in the intestines. The scientists were able to visualize the presence of intrainestinal lipids using a dye. This enabled them to screen through a collection of worms that each had a different second mutation—in addition to the original genetic modification—for one that reversed the accumulation of intrainestinal lipids. They identified the *C. elegans* relative of the mammalian FOXO proteins, providing the first clue that FOXO is involved in the response to insulin.

Studies from Dr. Accili's laboratory and others showed that one specific FOXO protein—called FoxO1—integrates signals regulating β cell mass and stress response and responds differently to

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insulin and glucose. In most cell lines, FoxO1 can predominantly be found within the nucleus, the compartment containing the cell's DNA. In response to insulin, FoxO1 is quickly removed from the nucleus and inactivated as a result of its translocation to the area of a cell called the cytoplasm. In response to glucose, FoxO1 takes the opposite journey; any FoxO1 found in the cytoplasm is concentrated in the nucleus and localizes in a specific pattern. This shuttling of FoxO1 in response to hormones and nutrients allows it to be an effective sensor that relays the nutritional and hormonal status of an organ to the nucleus of a mammalian cell, enabling FoxO1 to alter which genes are turned on and off by controlling DNA transcription.

The Role of FOXO in β Cell Function

Because FoxO1 had characteristics of an important metabolic sensor, Dr. Accili was specifically interested in what happens to FoxO1 during development of diabetes. He and his colleagues took advantage of techniques that allowed them to visualize FoxO1 and insulin in the islets of mouse models of diabetes. First, they observed that FoxO1 was found only in β cells, not in any of the other cells found in islets. In healthy β cells, with normal glucose levels, FoxO1 and insulin co-localized to the cytoplasm of the cell. Because they observed FoxO1 in the cytoplasm, this indicated that it was inactive in healthy β cells. In early diabetes, with mild increases in the level of glucose, they found that FoxO1 relocated to the distinctive nuclear pattern in response to the stress, and insulin was less visible than it was in healthy β cells. As diabetes progressed and levels of glucose increased, both insulin and FoxO1 became even less visible, indicating that there is a tight correlation between progression of β cell dysfunction and levels of FoxO1. They did not know, however, whether the loss of FoxO1 was a cause or an effect of β cell failure, nor what happened to the cells that were producing insulin.

To learn more about the role of FoxO1 in β cell failure, Dr. Accili and his colleagues genetically modified mice to lack FoxO1 specifically in their β cells (while leaving it intact in other parts of the body). Again, they used techniques to visualize insulin and also glucagon, a hormone produced by the pancreas that signals the liver to release stored glucose. Under standard conditions, mice lacking FoxO1 in their β cells appeared relatively normal. They displayed normal glucose tolerance and insulin and glucagon secretion. To assess the consequences of loss of FoxO1 in the response to metabolic stress, the scientists looked at the FoxO1-deficient β cells of female mice that had undergone multiple pregnancies (multiparous) and of aging male mice. Intriguingly, in these mice, the FoxO1-deficient β cells underwent a similar process as the diabetic β cells, suggesting that loss of FoxO1 could be a cause, rather than a consequence, of β cell dysfunction. Under both of the metabolic stress conditions, they observed increased glucose levels, impaired glucose tolerance, decreased insulin secretion, and increased glucagon secretion. These conditions—elevated glucose and glucagon levels and low insulin—mimic conditions found in humans with type 2 diabetes, suggesting that the β cell dysfunction in FoxO1-deficient mice could be similar to that in people with diabetes.

Because only the β cells had been altered in these mice, the resulting elevated glucose and glucagon levels and low insulin are not due to insulin resistance; cells that respond to the activity of insulin had not been modified. Therefore, the scientists could probe why β cells without FoxO1 failed in the absence of insulin resistance. To determine whether the loss of insulin production was due to death of the β cells or to loss of their capacity to carry out their normal function, Dr. Accili and his colleagues performed a “lineage tracing experiment” where, using the previously

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described mice lacking FoxO1 in their β cells, they labeled the β cells without FoxO1 with one color chemical label and insulin-containing cells with another color label. β cells that lacked FoxO1 but could still produce insulin would thus have both color labels. This experiment allowed the scientists to draw conclusions about what happens to the FoxO1-deficient β cells when animals have undergone metabolic stress, such as multiple pregnancies. For example, if the FoxO1-deficient β cells die, they would expect to see no cells labeled with the color that marks FoxO1 deficient cells.

In virgin mice, Dr. Accili and his colleagues observed that FoxO1-deficient and normal islets looked similar, suggesting that, without metabolic stress, mice with FoxO1-deficient β cells have a similar endowment of β cells and that these β cells function properly. Following multiple pregnancies, the islets of the FoxO1-deficient mouse—which had since developed diabetes—looked different from the islets of a multiparous, normal mouse. Unexpectedly, they observed an increase in the number of FoxO1-deficient cells without insulin. Thus, the FoxO1-deficient β cells did not die, but instead lost the ability to produce insulin.

New Identities for β Cells in the Absence of FOXO

To understand more fully the β cell failure in these FoxO1-deficient cells, Dr. Accili and his colleagues looked at important markers of β cell identity and function, including factors called MafA and Pdx1. They observed that these markers were absent in the cells of FoxO1-deficient mice that no longer produced insulin. Because these markers are important to β cell development, Dr. Accili and his colleagues wondered if these FoxO1-deficient cells had lost even earlier markers of β cell identity.

They focused on a factor called Neurogenin3 (abbreviated Neurog3), which is found in endocrine precursor cells. Neurog3 is a marker shared in common by cells destined to develop into different endocrine cell types. This is an important stage, therefore, because a cell making Neurog3 has made a decision to become an endocrine cell, but has not yet committed to which endocrine cell type it will become. Because this stage occurs prior to birth in mouse and human, the adult pancreas contains few, if any, cells with Neurog3. In multiparous mice that lack FoxO1 in their β cells, the scientists observed a number of cells with Neurog3. They also observed the appearance of Neurog3-containing cells in aging male mice. Cells with high levels of Neurog3 contained no insulin, Pdx1, and MafA, while normal mature β cells showed the opposite pattern. This important result suggests that a developmental stage—when precursor cells are marked by Neurog3—that was previously thought to be very transient and never reproduced in the life of an adult β cell, occurs when adult β cells lack FoxO1. In addition to Neurog3, other auxiliary markers that normally accompany Neurog3 were present as well. This indicated that FoxO1-deficient β cells revert to an uncommitted precursor-like stage under conditions of metabolic stress.

Another aspect of type 2 diabetes is that people with the disease have high levels of glucagon, so Dr. Accili and his colleagues wondered whether the reversion of FoxO1-deficient β cells to a progenitor-like state was associated with potential for conversion to another pancreatic cell type. When they visualized insulin and other pancreatic hormones (like glucagon) in a normal islet, they did not observe cells that contained both insulin and other hormones known to be made by other types of islet cells. In the absence of FoxO1, however, they observed these other hormones in cells that used to be β cells (before the loss of FoxO1 following exposure

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to metabolic stress). This observation suggests that the FoxO1-deficient cells had lost their β cell identity and had taken on a new identity—producing other pancreatic hormones. This provides an explanation for the high levels of glucagon seen in FoxO1-deficient, multiparous mice and suggests that high levels of glucagon seen in people with diabetes might be explained, in part, by cells losing their β cell identity and taking on a new, glucagon-producing identity. None of the converted cells produced insulin as well, consistent with the idea that regression of FoxO1-deficient β cells to a distinctive pre- β cell state is a prerequisite for their conversion into a new identity.

To ask whether these observations were limited to experimentally induced deficiency in FoxO1 or whether they represented a common path to β cell failure, the scientists looked at mice that spontaneously developed diabetes and observed that, although insulin levels decreased as glucose levels increased, the β cells did not die. Rather, in the diabetic mice the β cells became marked by Neurog3 and its accompanying factors and appeared to lose FoxO1. Therefore, it appears that, in mice, FoxO1 is required to enforce β cell identity in insulin-resistant diabetes and suggests that gradual loss of FoxO1 during diabetes progression leads to loss of β cell identity and causes the pathogenic β cell failure observed in type 2 diabetes. Dr. Accili and his colleagues are beginning experiments to determine whether the same process occurs in humans.

Conclusions and Implications

Dr. Accili's research indicates that loss of β cell identity, not β cell death, is a key feature of type 2 diabetes. This is a departure from the widely held view that β cell failure is caused by a reduction of β cell mass following cell death. Rather, this research shows that β cells lose their ability to produce insulin and gain a new identity and the ability to produce glucagon, and that this

process is caused by loss of FoxO1 function. FoxO1 is required to maintain β cell identity and prevent conversion into other pancreatic cell types in response to chronic metabolic stress. Dr. Accili proposed that FoxO1 carries out these critical functions by promoting genes required for β cell identity and by preventing reactivation of precursor genes.

Dr. Accili suggested that these new results could lead to improved approaches to treat type 2 diabetes. However, the current research emphasis to develop new therapeutics that increase production of insulin from remaining β cells or that induce β cell replication do not address this critical problem. Treatments that salvage cells that have regressed to become progenitor-like cells and restore them to become β cells again could be fruitful, taking advantage of the fact that the cells are still alive and present. Allowing metabolically stressed β cells to rest may be key to such an approach. Clinical studies have suggested that treating newly diagnosed patients aggressively with insulin, either with a pump or multiple daily injections, induces a remission of type 2 diabetes such that normal fasting glucose levels are maintained for some time after insulin is withdrawn. Other diabetes medicines do not achieve such prolonged effects after they are discontinued, and insulin is only able to do so early in the course of type 2 diabetes. In other words, providing insulin to meet the demand on β cells could allow β cells to “rest” and result in a window of opportunity to intervene in the progression of the disease. Dr. Accili put forth that, once ways are devised to coax former β cells back to a β cell identity, coupling this approach with intensive insulin treatment at disease onset could become an improved strategy for treating type 2 diabetes.

*Talchai C, Xuan S, Lin HV, Sussel L, and Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* 150: 1223-1234, 2012.*

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Anastasia Albanese-O'Neill

Research To Combat Type 1 Diabetes: It's All in the Family



Anastasia Albanese-O'Neill, with husband Dan and children Cassidy and Jackson

In 2002, little did Anastasia Albanese-O'Neill know that her personal and professional life would change because of an unexpected diagnosis. It was that year that her daughter, Cassidy, was diagnosed with type 1 diabetes at 16 months of age.

Type 1 diabetes is an autoimmune disease in which the immune system destroys cells in the pancreas that make insulin. People with the disease must carefully monitor blood sugar levels and administer insulin, either through injections or an insulin pump.

"We didn't know anything about diabetes," recalls Anastasia. "No one else in our family had the disease." Cassidy was diagnosed with type 1 diabetes in the hospital and admitted to the intensive care unit (ICU)

because her blood sugar levels were dangerously high. "In the ICU we learned that there was no cure for type 1 diabetes and that Cassidy would need multiple daily insulin injections and 8 to 10 finger pricks every day to monitor blood sugar levels. We were sent home with a box of medical supplies and, to be honest, a sense of dread."

Even under those life-changing circumstances, she and her husband, Dan, recognized the importance of research to combat type 1 diabetes and enrolled Cassidy into a research study. Since that time, the family has continued to make major contributions to type 1 diabetes research.

Participating in the SEARCH for Diabetes in Youth Study

"While we were in the hospital, we were asked to participate in a study called SEARCH for Diabetes in Youth," Anastasia remembers. SEARCH is a joint effort of the NIDDK and the Centers for Disease Control and Prevention, and receives support from the *Special Statutory Funding Program for Type 1 Diabetes Research*. SEARCH was started to provide much-needed information on how many children and youth under age 20 in the United States have diabetes and how those rates were changing over time. That information is critical for informing public health efforts to fight the disease.

Although it was daunting for Anastasia and Dan to find out that their young daughter had type 1 diabetes, they were eager to sign up for the SEARCH study. "It was an

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easy study to participate in—we just needed to share some of our daughter’s medical and demographic information, and so we enrolled,” she explains.

And their effort has paid off. Because of SEARCH, which is conducted by researchers at multiple centers around the country, the Federal government now has an estimate of how many children and youth in the United States have diabetes: an estimated 15,600 youth are diagnosed with type 1 diabetes each year and an estimated 215,000 youth had diabetes (type 1 or type 2) in 2010. SEARCH has also found that rates of type 1 diabetes in youth under age 20 have increased by a surprising 23 percent between 2001 and 2009. Exact reasons for the increase are not known, but researchers speculate that there is something in the environment that is triggering the disease. A question remains as to what the environmental trigger, or triggers, may be—knowledge that is critical to finding ways to prevent type 1 diabetes. Anastasia and her family are also helping to answer that important question.

“I can tell you with absolute certainty that the course of Cassidy’s life so far, along with her future prospects, are indisputably better because of medical research,” says Anastasia.

Participating in the TEDDY Study

In 2005, Anastasia and Dan welcomed their second child, Jackson. Before Jackson was born, the family found out about another NIDDK-led research study, called The Environmental Determinants of Diabetes in the Young, or TEDDY. This international study was seeking to enroll and follow newborns to identify environmental triggers of type 1 diabetes. Anastasia and Dan allowed researchers to test Jackson’s blood shortly after he was born to see if he was at

increased genetic risk for developing type 1 diabetes. “Unfortunately, he was,” his mom recalls, which made him eligible to participate in TEDDY.

Anastasia and Dan next had to decide whether or not to enroll their son into the study. “My husband and I—after much discussion—agreed to participate,” explains Anastasia. “It was a tough choice. The study required a 15-year commitment from our family.” Jackson became one of the first children enrolled in TEDDY, and is now one of over 8,000 children being followed until they are 15 years old to collect data on their infectious, dietary, and other exposures and life experiences toward identifying an environmental trigger. TEDDY also is supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*.

Identifying environmental factors is much like looking for a needle in a haystack because there are so many factors that could be the culprit. Thus, “the list of data collected on each child is extensive,” explains Anastasia. “We provide samples of our water, we send in toenail clippings, we keep track of what Jackson eats and when he was immunized. We even overnight [mail] stool samples.” They’ve become so used to doing this, “I don’t get nervous at the post office when they inevitably ask, ‘What’s in the box, Ma’am?’ If Jackson is along, he answers for me,” she says with a laugh.

Jackson has been a real trooper, especially during the blood draws—the toughest part for him. “Each time we go to an appointment, we ask him if he would like to skip the blood draw, but he’s only asked not to do it once,” Anastasia reports. “He always replies that he needs to do it for Cassidy.” She explains that her son experiences the day-to-day challenges of living with type 1 diabetes and sees this study as his way of helping. “I’m really proud of him,” says his mom.

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TEDDY has the potential to revolutionize the ability to prevent type 1 diabetes. For example, identification of a dietary or infectious cause could have an enormously positive impact on public health through a diet change or vaccine for disease prevention. Therefore, Jackson is helping countless future generations of children who may be spared from type 1 diabetes because of his and his family's dedication to research. "If we can find a way to prevent the disease, it will be worth all the effort," says Anastasia.

A Career Change to Diabetes Research

One morning in 2007, Cassidy asked her mom an unexpected question. "I was driving my daughter to school when she was six, and she asked from the back seat, 'Mommy, what are YOU doing to cure diabetes?'," Anastasia recalls. "I don't remember exactly how I responded, but I know that later that week I enrolled in a chemistry class as a prerequisite to nursing school," even though she was working as a marketing director for a community college at the time and had no medical background.

In 2008, Anastasia received her nursing degree, and began working part-time in a research laboratory. During that time, she also had the opportunity to work in a pediatric diabetes clinic as a nurse educator, where she worked with families of children with diabetes. As a parent of a young child with the disease, she was able to relate to those families and felt that she could help them. That feeling prompted her to take her training a step further and enroll in a graduate program focusing on the use of technology in diabetes care and management. "I am working toward becoming a nurse practitioner and getting a Ph.D. in nursing because I want to do research to help families have better technologies," she explains. She is currently pursuing those studies full-time, while continuing to work part-time in the clinic. This is

another example of how she is making far-reaching contributions to type 1 diabetes research.

A Family's Hope Through Research

Although she is busy raising two young children, participating in an intensive research study, pursuing graduate studies, and working in the clinic, Anastasia makes time to advocate for research on type 1 diabetes. For example, she is serving a 2-year term on the American Diabetes Association's National Advocacy Committee. Why does she do it? "Without research, there is no hope for prevention, for a cure, or for better treatments. For our family, it's simple. Research equals hope," Anastasia exclaims.

She also says that the importance of research is evident when she looks at her daughter. Cassidy started using an insulin pump when she was 2 years old and also uses other devices, such as blood sugar meters that give a near-immediate reading. These technologies allow her to keep her blood sugar at almost normal levels, which is important because research has shown that good blood sugar control greatly reduces risk for the development of long-term diabetes complications. "I can tell you with absolute certainty that the course of Cassidy's life so far, along with her future prospects, are indisputably better because of medical research," says Anastasia.

"Without research, there is no hope for prevention, for a cure, or for better treatments. For our family, it's simple. Research equals hope," Anastasia exclaims.

However, that in no way means that managing type 1 diabetes is easy. It is a daily struggle: "We cannot get through a day without having to answer to it—it is unrelenting," says Anastasia. "I think the hardest part

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is that diabetes takes away the spontaneity, freedom, and innocence of being a child. If Cassidy is going to a sleepover or a birthday party, we have to have a discussion with the other parents about diabetes. If she is going for a swim, she has to worry about her insulin pump being in the sun and whether her blood sugar will be too high or too low while in the water. As a mom, I want really badly for my child not to have to deal with those kinds of problems and to have the freedom to just be a kid.” Those are just a few reasons why finding a cure is so important. “The day that a cure is discovered will be a day to rejoice.”

In the meantime, Cassidy and her family are vigilant about managing her type 1 diabetes. And the proud

mom is happy to report that, “My daughter is a happy, healthy 11-year-old in the sixth grade, who dances and plays volleyball and does well in school.”

Jackson, 7 years old and in the second grade, is a competitive gymnast and also does well in school. He remains an active participant in the TEDDY study. “The good news is that he does not have diabetes,” his mom notes happily.

As for Anastasia, her dedication to type 1 diabetes research is unwavering: “I plan to retire within minutes of the discovery of a cure for type 1 diabetes, but not a moment earlier.”

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Jameel Shareef

TODAY's Lifestyle for Tomorrow's Health with Type 2 Diabetes



Jameel Shareef

Twenty-year-old Jameel Shareef describes himself as a funny, humorous, spontaneous guy who likes to make people laugh. He loves football, and his life's ambition is to become a football analyst for a major cable news sports network.

Diagnosed with type 2 diabetes at age 13, Jameel knows that if he's going to be successful at achieving his life's dream, he's also going to need to succeed at managing his diabetes.

Jameel and Type 2 Diabetes

Currently a college student, Jameel first noticed something wasn't normal when he was in middle

school, during an hours-long bus trip to watch a football game at Giants Stadium, near New York City. "During that trip, I was constantly thirsty," says Jameel, "and on the ride home I was using the bus bathroom every 5 or 10 minutes."

Things got progressively worse. "When I started school that September, in 2005, I felt tired and looked pale, my skin complexion started to change, and I constantly was drinking orange juice," he says. His mother reacted quickly and took him to a nearby medical center, where he was immediately diagnosed with type 2 diabetes.

"The same month as my diagnosis, a nurse brought the TODAY (Treatment Options for Type 2 Diabetes in Adolescents and Youth) study to my attention and strongly encouraged me to enroll," says Jameel. "I was told it was good I was diagnosed early so that I can control my diabetes and stay healthy." With his parents' consent and support, Jameel enrolled in the study.

Type 2 diabetes was previously called "adult-onset" diabetes because it was predominantly diagnosed in older individuals. In the mid-late 1990s, however, doctors began increasingly to see type 2 diabetes in adolescents. Over the years since, and believed to be related to the childhood obesity epidemic, there has been a dramatic rise of type 2 diabetes in young people like Jameel. The SEARCH for Diabetes in Youth study, a joint effort of the NIDDK and Centers for Disease Control and Prevention, reported that in the United States, an

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estimated 3,600 young people develop type 2 diabetes each year, with youth from racial/ethnic minority groups disproportionately affected.

“...it was good I was diagnosed early so that I can control my diabetes and stay healthy,” says Jameel.

With the rise of type 2 diabetes in youth comes another serious problem. The longer a person has type 2 diabetes, the greater the likelihood that they will develop complications from the disease, including heart disease, stroke, eye disease, nerve damage, and kidney disease. This makes it critical for young people with type 2 diabetes to achieve quick and sustained control of their blood sugar, which is why Jameel’s enrollment in the TODAY study early in the course of his disease was important.

About the TODAY Study

Because type 2 diabetes had been primarily an adult illness, information about how to effectively treat young people with the disease was limited, leaving pediatric diabetes experts to rely on what was known about how to treat adults with the disease. The TODAY study set out to determine how best to treat children and adolescents. This study was conducted by researchers at 16 medical centers around the country, and funded by NIDDK. The study enrolled young people who had been diagnosed with type 2 diabetes for less than 2 years, and had a body mass index (BMI) at the 85th percentile or greater. BMI is a measurement of weight in relation to height. In children and adolescents, overweight is defined as a BMI at the 85th to 94th percentile for their age and sex; obesity is defined as a BMI at the 95th percentile or more. At the time of his diagnosis, Jameel was 5 feet 9 inches tall and weighed 234 pounds, which placed his BMI in the

obese category for youth, and he was eligible to enroll in the TODAY study.

The study enrolled nearly 700 young people between the ages of 10 and 17, who agreed to participate for 2 to 6 years. Each TODAY study participant was randomly assigned to one of three treatment options: (1) the diabetes medication metformin; (2) metformin plus another drug, rosiglitazone; or (3) metformin plus an intensive lifestyle program that coordinates nutrition, physical activity, and behavior modification. Metformin is a widely used, first-line treatment for adults with type 2 diabetes. Currently, metformin is the only oral drug approved by the U.S. Food and Drug Administration (FDA) for treating type 2 diabetes in youth ages 10 to 17.

In April 2012, TODAY study researchers reported that metformin alone was inadequate for maintaining acceptable, long-term, blood sugar control in over 50 percent of youth. Adding the lifestyle intervention to metformin provided no more benefit, overall, than metformin therapy alone. This suggests that type 2 diabetes may progress more rapidly in young people than it does in adults, and that it may be best to start with a more aggressive drug treatment approach in youth with type 2 diabetes. The combination of metformin and rosiglitazone was more effective in treating young people with recent-onset type 2 diabetes than metformin alone. Importantly, however, after the study began, the FDA restricted use of rosiglitazone because of studies linking the medicine to a higher risk of heart attacks and stroke in adults.

Lifestyle Changes Are a Touchdown for Jameel

Jameel received the treatment of metformin plus an intensive lifestyle program. The TODAY lifestyle intervention was a family-based weight-management program that included intensive education and

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activities delivered one-on-one by trained study staff. While the overall study results didn't show any additional benefit from the lifestyle intervention, Jameel has personally benefitted from taking part in the lifestyle program. "I had to meet with my TODAY coach every couple of weeks. She taught me how to stay healthy by teaching me how to identify and separate good foods from foods that are bad for me, how to manage the size of my meals, how to hydrate myself and cut soda out of my diet, and how to exercise," explains Jameel.

At the time of his enrollment in the TODAY study, Jameel was in middle school and dealing with being overweight. "It was a big deal telling my friends that I had type 2 diabetes. I was uncomfortable, at first, but the coaching helped me to get over my discomfort. The coaching also motivated me, as well as allowed me to talk about things that were personal to me as an adolescent and young adult."

Seven years later, Jameel is still active in the TODAY study. He is now 6 feet tall and weighs 240 pounds, "mainly muscle mass," he says, because of all his exercising and the free weights he continues to use. He adds with a smile, "many of my classmates think I am a member of the university's football team." Jameel continues to see his TODAY coach about every 3 months. At each meeting, his hemoglobin A1c (an indicator of a person's blood sugar levels over the previous 2 to 3 months), height, weight, and blood

pressure are measured. He also provides TODAY researchers with an overview of his exercise workout and eating habits. He is still taking metformin.

"Taking part in the TODAY study has been a great experience," says Jameel. "My coach motivated me and made me want to stay healthy. I would greatly encourage other young people to take part in a study like this."

Jameel reports, "I feel great. The TODAY study taught me how to eat and exercise properly and how to maintain my diabetes from a young age. As a result, I'm proud to say I've never been hospitalized nor had any complications as a result of my type 2 diabetes."

"Taking part in the TODAY study has been a great experience," says Jameel. "My TODAY coach motivated me and made me want to stay healthy. I would greatly encourage other young people to take part in a study like this."

Staying healthy with what he has learned in the TODAY study and motivated by his education and career ambitions, Jameel's future looks very bright.

For more information about the TODAY study, please see the related advance in this chapter.

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Rachel Bunn

Battling Cystic Fibrosis-related Diabetes by Participating in a Clinical Trial



Rachel Bunn with her husband Josh

Rachel Bunn was born 2 months premature, and diagnosed with cystic fibrosis (CF) at the time of her birth. CF is a genetic disorder that results in thick mucus and abnormal sweat, which leads to frequent, serious lung infections, as well as severe complications of the pancreas, liver, digestive tract, and other organs. In years past, children diagnosed with the disease had a predicted median survival of about 10 years, with few surviving beyond their teens. The good news is, due to new treatments made possible by medical research, many people with CF now survive to enjoy many fulfilling years of adulthood.

Unfortunately, as they age, an increasing number of people with CF are developing cystic fibrosis-related diabetes, or CFRD, a form of diabetes that develops as a result of damage to the insulin-producing cells of the pancreas. Rachel learned she had

CFRD in her 20s. However, her participation in an NIDDK-supported clinical trial on CFRD has helped determine how she and others with CFRD can control the disease and live longer, healthier lives.

Living with Cystic Fibrosis

Rachel has been beating the odds her whole life. She required emergency surgery in her first few days of life to remove an intestinal blockage, a common complication in newborns with CF. Her weight fell to just three pounds, and her health care providers were not optimistic. “At the time, they said I wouldn’t survive,” says Rachel.

But she did survive, and has continued to battle CF and its complications, including the intestinal obstructions and lung infections, ever since. While normal mucus is watery thin, keeping the lungs healthy and allowing for efficient movement of air, CF causes mucus to be very thick and sticky, increasing risk of serious infection and impeding air flow. As Rachel puts it, “the easiest way to describe what it’s like to live with cystic fibrosis is that my lungs are like glue inside instead of like water. When I get infections, the mucus secretions are so thick I can’t fight them off like other people do.” Instead, Rachel is prescribed powerful antibiotics. If oral medicines don’t work, the antibiotics are administered intravenously through PICC lines (peripherally inserted central catheters) to fight off the infection. Rachel says she averages one or two PICC line treatments a year, which last for 2 to 3 weeks at a time. “They’re not fun,” she says.

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“The easiest way to describe what it’s like to live with cystic fibrosis is that my lungs are like glue inside,” says Rachel. “When I get infections, the mucus secretions are so thick I can’t fight them off like other people do... Sometimes it feels like there’s a rubber band around my lungs, and breathing gets difficult.”

In addition to the infections, Rachel has suffered numerous intestinal blockages. One particularly bad blockage occurred 3 months after she was married. “My intestine wrapped around itself, and I was in intense pain,” says Rachel. She was rushed to the hospital and underwent 8 hours of emergency surgery, during which surgeons removed more of her intestines.

To maintain her health as best she can, Rachel does a daily regimen of lung therapy, which includes the use of a percussion vest. “The vest helps break up the excess mucus in my lungs,” reducing risk of infections, explains Rachel. She also says that diet and exercise help to keep her lungs clear, so she tries to run or swim daily, which is challenging. “I often get tired real easily,” she says, “Sometimes it feels like there’s a rubber band around my lungs, and breathing gets difficult.”

About CFRD

In addition to the damaging effects of CF on the lungs, CF interferes with pancreatic function in two important ways, both of which can have the effect of causing serious weight loss. From infancy, the thick mucus of CF patients blocks secretion of pancreatic digestive enzymes needed for absorption of nutrients by the digestive tract and for normal growth. Today, fortunately, babies are screened for CF at birth, and can obtain an adequate supply of these enzymes by taking them in medication form with their meals. Early

intervention allows most people with CF to achieve near normal growth.

Indeed, thanks to this and many other improvements in treatment, more and more people with CF are reaching adulthood, which is when the second major pancreatic complication often arises: for reasons that remain unclear, CF can lead to a decline in the capacity of the pancreas to supply the body with insulin, the hormone needed to transport glucose (sugar) into cells. At the same time, also for uncertain reasons, the rest of the body may become less sensitive to insulin. The net result, CFRD, leads to loss of body weight and muscle mass in people who are underweight to begin with. In addition, CFRD increases the decline in lung function, and reduces survival. More research is needed to understand how CFRD affects the function of other organs involved in CF.

Importantly, although CFRD has features in common with type 1 and type 2 diabetes, it is distinct from both. Unlike type 1 diabetes, the insufficient insulin production in CFRD stems not from an autoimmune attack on the pancreas, but rather from a progressive loss of pancreatic function similar to what is seen in type 2 diabetes. And while CFRD involves insulin resistance and has other metabolic and genetic similarities to type 2 diabetes, it is not associated with being overweight or obese.

Indeed, when CFRD was first recognized, clinicians were concerned about its tendency to induce weight loss in CF patients, who are often underweight already. However, it was unclear whether people with the disease were likely to face the same array of other serious complications endured by people with more common forms of diabetes, and which are a major reason for early, aggressive therapy to lower blood sugar in type 1 and type 2 diabetes. Many health care providers

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were therefore reluctant to prescribe insulin for CFRD, because no one knew whether insulin, or indeed any drug used to treat other forms of diabetes, would help people with CFRD to be healthier. Furthermore, its use is an additional burden on the patient, and an accidental overdose could cause dangerous hypoglycemia.

Research Improves Treatment for CFRD

To provide answers to these important questions and ascertain the benefits and risks of different CFRD treatment options, the NIDDK supported a clinical trial called the Cystic Fibrosis-Related Diabetes Therapy Study (CFRDT Study). Study participants were randomly assigned to one of three groups: 1) to inject pre-meal insulin; 2) to take an oral medication called repaglinide, which is approved for treatment of type 2 diabetes and acts by stimulating the pancreas to secrete more insulin; or 3) to take an oral placebo (an inactive pill given for comparison against the real medication being tested). Participants taking a pill did not know whether they were taking repaglinide or placebo.

Rachel was actually diagnosed with CFRD as a result of being screened for the study, and she was understandably worried. The daily regimens required to control either CF or diabetes are onerous on their own. The prospect of managing both was truly daunting. But she enrolled, recognizing the importance of the study, and was assigned to one of the study arms that received pills. Thanks to her and the other participants, the CFRDT Study was able to demonstrate conclusively that insulin therapy indeed can help people with CFRD maintain their body weight, improve lung function, and feel healthier.

After the results of the study were known, Rachel began to take insulin, which she now administers to herself via an insulin pump. When she was first approached about using a pump, “I thought they were

only for people with severe diabetes,” she recalls. But, she is happy to report that the insulin pump “changed my life dramatically.” While she still has the lung infections and digestive issues associated with CF, her CF-related diabetes has improved. “Thanks to the insulin pump my diabetes is well under control,” she explains, and she’s on the least amount of insulin that can be administered through the pump.

Based on the results of the study, Rachel began to take insulin, which she now administers to herself via an insulin pump. She is happy to report that the insulin pump “changed my life dramatically.” Today, Rachel says, “I look and feel the best I’ve ever felt.”

Insulin treatment has also improved her body weight. Prior to entering the study, her body mass index (BMI), a measure of weight in relation to height, was below the normal range. After starting insulin therapy, however, her BMI has gradually risen into the low 20s (normal BMI is between 20-25), which is to say, a very healthy weight. Today, Rachel says, “I look and feel the best I’ve ever felt.”

Living Life to the Fullest

Not one to feel sorry for herself because of her CF, or her CFRD, Rachel decided instead to take charge of her life. Now 30 years old, she’s been married for 7 years to her husband Josh, an airline pilot; is self-employed as an independent agent for a major insurance company; and enjoys the love and support of family. “I feel extremely fortunate,” says Rachel.

Rachel is eager to share her story about benefitting from research, and has done so at different events, including at her local chapter of the Cystic Fibrosis Foundation. “I feel the need to speak up because there just aren’t enough

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CF patients my age who can do it. Many are on oxygen or have had lung transplants.” There’s no doubt that Rachel has reacted to her health challenges positively, courageously, and with a desire to help others. When people ask her how she deals with these challenges, Rachel’s response is: “10 percent of life is what happens to you; 90 percent is how you react to it.”

Rachel is eager to share her story about benefitting from research. “I feel the need to speak up because there just aren’t enough cystic fibrosis patients my age who can do it.”

Hope Through Research

The NIDDK is bolstering research on CFRD, to understand its causes and consequences. For example, research will address why some people with CF develop CFRD and others do not, and also examine the molecular mechanisms by which CFRD contributes to a steeper decline in lung function compared to CF patients without diabetes. This knowledge could pave the way toward new prevention and treatment strategies. The NIDDK also continues to support research on CF, including research to develop new therapies, to help Rachel and others with CF live longer, healthier lives, and reduce the burden of managing the disease.