Recent Scientific Advances

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GOAL V:
PREVENT OR REDUCE THE COMPLICATIONS OF TYPE 1 DIABETES
For a child at the beginning of the 20th century, the diagnosis of type 1 diabetes was equivalent to a death sentence. The discovery of insulin commuted that death sentence, but it soon became apparent that insulin treatment allowed many of these children to live just long enough to develop diabetes-induced blindness, kidney failure, and coronary disease. At the beginning of the 21st century, type 1 diabetes still has a profound effect on many people’s lives, because these long-term health complications that affect nearly every organ system in the body still occur with alarming frequency.

Patients with type 1 or type 2 diabetes face the possibility of similar complications. For patients and their families, the following grim statistics about complications are far too familiar.

- Life expectancy may be shortened by about 15 years (4), with premature deaths due primarily to heart attacks and strokes (5). Rates of cardiovascular disease are increased up to 10-fold compared to people in the age-matched general population (2, 3). In addition, diabetes impairs repair pathways necessary for the success of established cardiovascular therapies, such as coronary angioplasty, bypass grafting, and lower extremity revascularization. This causes a significantly lower success rate in treating vascular diseases in patients with diabetes.

- In addition to injuring the large vessels of the heart and brain, diabetes damages the small blood vessels of the body, called the microvasculature. Diabetes (type 1 and type 2) is now the leading cause of new blindness in people 20 to 74 years of age (5). Retinal damage (retinopathy) is detectable in virtually all patients with type 1 diabetes after 20 years, as a consequence of damage to the microvessels of the retina.

- Diabetes also leads to protein in the urine (microalbuminuria) and eventually to irreversible kidney disease (nephropathy), which progresses to end-stage renal disease, requiring dialysis or kidney transplantation. Importantly, only about one-third of patients with type 1 diabetes appear to be susceptible to this devastating complication (20). The factors that determine susceptibility or resistance to nephropathy are still unknown.

- More than 60 percent of patients with diabetes are affected by painful nerve damage and loss of sensation (neuropathy), particularly in the legs and feet (5). Foot ulcers often arise because of the inability to perceive pain, and then fail to heal because of insufficient blood flow and other factors secondary to diabetes. Amputation of the lower extremities is too frequently the end result of nonhealing ulcers. Diabetic nerve damage also contributes to erectile dysfunction, urinary incontinence, and nocturnal diarrhea.

- Depression severe enough to warrant intervention is increased in patients with type 1 diabetes. Conventional antidepressant treatments are effective in the presence of diabetes, but with discontinuation of treatment, recurrence of depression is common and usually accompanied by deterioration in glycemic control.

- Other complications of type 1 diabetes include increased rates of birth defects in children of mothers with diabetes and severe periodontal disease.

Preventive strategies are beginning to reduce the incidence of diabetes complications. Nonetheless, tight control of blood glucose levels is difficult to achieve because of the risk of hypoglycemia and the need for unrelenting vigilance about dietary intake, the monitoring of blood glucose levels, and the administration of insulin. Good control of blood pressure and blood lipid levels—as well as careful monitoring for retinal damage, albumin in the urine, sores on the feet, and other signs and symptoms of diabetes—are all measures for preventing or mitigating complications of diabetes. Although blockade of the renin-angiotensin system is well-established to slow the progression of diabetic nephropathy, it does not always prevent the development of renal failure. The treatment options for other complications are even less satisfactory. In particular, symptomatic treatment for nerve pain is poor. While laser photocoagulation has been an extremely
important advance for treating diabetic retinopathy, vision loss from this complication is not always preventable, even with the best interventions.

In type 1 diabetes, complications are due to metabolic derangements caused by loss of insulin resulting from the autoimmune destruction of insulin-producing beta cells. The best understood metabolic defect causing diabetic complications is hyperglycemia, which can modify the extracellular environment and, in some cell types, directly lead to excess glucose inside the cell. Intracellular hyperglycemia initiates a cascade of changes in cell metabolism that includes increased production of reactive oxygen species, increased levels of sugar-modified proteins, and activation of a number of signaling pathways. In addition to causing hyperglycemia, insulin deficiency can contribute to end-organ damage in type 1 diabetes through alterations in the metabolism of lipids and lipoproteins. In the endothelial cells that line the blood vessels in the eye, kidney, nerve, and heart, these alterations lead to increased leakiness of blood vessels, decreased vascular density, inadequate delivery of blood due to cell loss, and altered expression of cell-surface proteins that initiate and perpetuate a damaging inflammatory response. Tissue responses to these cellular changes in the blood vessels are specific for each tissue and organ, with genetic variation playing an important role in determining the nature and extent of these responses for each individual.

Advancing scientific knowledge of the physical and emotional complications of diabetes to improve clinical care is a multidimensional challenge. Meeting this challenge would have overwhelming benefits for people with type 1 diabetes. New discoveries about diabetic complications may also improve the lives of millions of Americans with type 2 diabetes, who also suffer from the same devastating complications.

**Recent Scientific Advances**

Significant progress in understanding diabetic complications has occurred in the past decade. These discoveries are leading to the development of effective therapies to prevent and treat the cell, tissue, and organ damage caused by diabetes.

**Progress in Reducing Diabetic Nephropathy:** Recent reports indicate that prevention efforts are beginning to have dramatic effects on the rates of diabetic nephropathy in patients with type 1 diabetes. This devastating complication of diabetes has historically been seen in as many as one-third of individuals with diabetes after 20 or 30 years of disease (20). In the most recent population-based study from Finland, however, only 7.8 percent of patients with type 1 diabetes have renal failure after 30 years of diabetes (21). Declines in the incidence of end-stage renal disease due to diabetes are being noted for the U.S. population as well, in reports from the United States Renal Data System. These gains are most noteworthy in diabetic patients under age 30 (most of whom have type 1 diabetes) and are restricted to Caucasians and not observed in African Americans. The rate of end-stage renal disease in Caucasians under 30 with diabetes is nearly half the rate seen in the late 1980s and early 1990s (9). Since that time, several clinical strategies have been proven to significantly reduce the progression of diabetic nephropathy. These include angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin receptor blockers (ARBs), which lower protein in the urine and are thought to directly prevent injury to the kidneys’ blood vessels; and careful control of blood glucose and blood pressure. Credit for the recent improvements likely goes to implementation in clinical practice of these strategies to prevent disease, including better glycemic control, hypertension management, and use of ACE inhibitors and ARBs. Research to build on this success and extend it to all populations in the United States is a high priority for the NIH.

**Role of Reactive Oxygen Species (ROS) in Complications Pathogenesis:** Over the past 35 years, several molecular mechanisms have been implicated in glucose-mediated vascular damage. Each of these mechanisms has been studied independently of the others, and there has been no apparent common element linking them. Recent discoveries have made clear that all of these seemingly unrelated mechanisms may arise from a single, hyperglycemia-induced process: the overproduction of the reactive free radical molecule, superoxide. It now appears that the energy-generating cellular organelles called mitochondria are required for the initiation of hyperglycemia-induced superoxide production, which can, in turn, activate a number of other superoxide production pathways that may amplify the original damaging effect of hyperglycemia. Increased free fatty acid oxidation in mitochondria produces superoxide as well. In diabetic mice genetically engineered to produce high levels of an enzyme that degrades superoxide (called “superoxide dismutase”), diabetes fails to activate any of the classic hyperglycemia-induced damaging pathways, and these mice do not develop...
diabetic kidney disease. This advance points to the central role of a single pathway involved with complications in multiple organs. Several novel pharmacologic approaches based on this unifying mechanism have already prevented diabetic eye, kidney, and nerve pathology in rodent models of diabetes.

Therapeutic Approaches Based on a Soluble Form of the Receptor for Advanced Glycation Endproducts (RAGE): Although elevated levels of low-density lipoproteins (LDL cholesterol) appear to be required for atherosclerosis, the pathologic result for any given level of LDL depends on a wide variety of other factors, many of which are pro-inflammatory molecules secreted by a variety of tissues and cell types. Recently, it has been established that nearly all people with type 1 diabetes have accelerated atherosclerosis and coronary artery disease, which is associated with increased local tissue levels and blood levels of pro-inflammatory molecules. A major advance in this field is the identification of RAGE, a component of the innate immune system originally identified based on its ability to bind sugar-modified proteins known as advanced glycation endproducts (AGE). RAGE is found in many tissues, including those prone to diabetic complications. The binding of AGE to RAGE activates signaling pathways that lead to generation of reactive oxygen species. In addition, a soluble form of RAGE binds a number of pro-inflammatory factors, such as S100 calgranulins and high mobility group box 1 (HMGB1), thereby preventing their interaction with cellular receptors. Treatment with this soluble form of RAGE prevents accelerated atherosclerosis in several models of experimental diabetes. In addition, soluble RAGE blocks kidney disease in diabetic mice. Because soluble proteins are not ideal pharmacologic agents in all settings, especially for those that require chronic administration over many years, additional research focused on the development of orally available antagonists (i.e., drugs that block the receptor), and modification of both the inflammatory peptides and the receptor, should lead to the development of important new therapies to retard atherosclerosis in people with diabetes.

Insulin Resistance—An Independent Cardiovascular Risk Factor in Type 1 Diabetes: In some regards, the classic distinction between type 1 and type 2 diabetes is beginning to break down. Type 1 diabetes is a disease in which there is a loss of insulin production due to autoimmune destruction of insulin-producing cells. In type 2 diabetes, loss of effective insulin action is due to a combination of defects, both in normal insulin action (insulin resistance) and in the ability of pancreatic beta cells to overcome this insulin resis-tance by secreting enough additional insulin. Over the past 10 years, evidence has mounted to show that, in type 1 diabetes, hyperglycemia itself eventually causes secondary insulin resistance in nearly all patients. Because insulin resistance appears to operate as a significant factor in the development of cardiovascular disease, independent of other mechanisms such as lipid abnormalities and high blood pressure, the discovery that hyperglycemia causes insulin resistance in type 1 diabetes links accelerated atherosclerosis in patients with type 2 diabetes with accelerated atherosclerosis in type 1 diabetes. In addition, insulin resistance leads to a greater likelihood of heart failure following a heart attack. These observations suggest novel pharmacological approaches for reducing cardiovascular disease in patients with type 1 diabetes via direct reduction of insulin resistance.

Diabetic Heart Damage from Altered Fuel Metabolism: A significant advance in understanding diabetic heart disease is the discovery that derangements in cardiac fuel utilization are, at least in part, responsible for myocardial disease caused by diabetes. Both cardiac and skeletal muscle cells (myocytes) have high energy requirements. Cells normally use both glucose and fatty acids as energy sources. In diabetic heart muscle, insufficient insulin action reduces the ability of myocytes to use glucose as a source of energy. To compensate, these cells switch to fatty acids as their primary energy source, with chronic activation of the nuclear receptor PPAR-alpha mediating this switch. The increased flux of fatty acids into heart muscle cells overwhelms the mitochondria’s abilities to burn this fuel. The result is intracellular accumulation of ROS and a variety of fatty acid metabolites, whose deleterious effects on cells have been termed “lipotoxicity.” Accumulation of fatty acid metabolites leads to death of cardiac myocytes in the hearts of diabetic animals and makes these cells especially sensitive to further damage from hypoxia associated with angina or a heart attack. These findings may lead to therapies that increase glucose utilization in heart muscle and thereby reduce the damage caused by accumulation of fatty acids. They could also lead to the development of diagnostic imaging methods to detect deleterious changes in cardiac cell metabolism before they lead to heart disease.

Impaired Blood Vessel Formation from Bone Marrow Progenitor Cells in Diabetes: Diabetic complications result not only from damage to cells and tissues, but also from the inadequacy of the repair process. During the acute response to injury, new blood vessel growth rescues “stunned” areas of the heart or central nervous system, reducing morbidity and mortality. With chronic low perfusion, the development of collateral vessels reduces the size
Circulating progenitor cells from the bone marrow promote the regeneration of blood vessels by acting in concert with the cells and extracellular matrix at the site of injury. A major advance is the observation that these endothelial progenitor cells are depleted and dysfunctional in diabetes, and that injection of normal progenitor cells can improve blood supply to the tissues and nerve function in experimental diabetes. Research focused on the diabetes-induced impairment of this process could lead to novel drug- and cell-based therapies for people with diabetes to restore compensatory vessel formation in cardiovascular disease, stroke, peripheral vascular disease, and wound healing. In the diabetic retina, however, overly exuberant vascular repair processes can result in excessive proliferation of small vessels. Molecular pathways responsible for the new vessel growth have been identified, and this work suggests new molecular targets for drugs that could protect the retina.

**Sustained Effect of Glycemic Control on Complications Susceptibility—“Metabolic Memory”:** In 1993, the results of the landmark Diabetes Control and Complications Trial (DCCT) showed that, in people with short-duration type 1 diabetes, intensive glycemic control dramatically reduced the occurrence and severity of diabetic microvascular complications. After the announcement of the DCCT results, many patients who had been in the standard therapy group adopted more intensive therapeutic regimens, and their level of glycemic control improved, as measured by the hemoglobin A1c (HbA1c) test. At the same time, the mean level of HbA1c worsened for patients who had been in the intensive therapy group. The post-DCCT HbA1c values for both groups have become nearly identical during the approximate 10 years of follow-up in the ongoing Epidemiology of Diabetes Interventions and Complications Study (EDIC).

Surprisingly and provocatively, however, the effects of a 6.5-year difference in HbA1c during the DCCT on the incidence of retinopathy and nephropathy have persisted, and have even become greater over the subsequent decade of follow-up. People in the standard therapy group continued to have a higher incidence of complications, even with an improvement in glycemic control during the EDIC. In contrast, people in the intensive therapy group continued to have a lower incidence of complications, even with a worsening of glycemic control during EDIC. In addition, early intensive glycemic therapy was recently shown to markedly reduce later development of atherosclerotic changes, heart attacks, and strokes.

The phenomenon that glycemic control could have long-lasting effects, called metabolic memory, elicits a number of questions: How can a finite period of good or bad glycemic control have such long-lasting effects? Is there a point in the development of complications in which the progression becomes relatively independent of glycemic control? The discovery of the molecular and cellular basis of metabolic memory is urgently needed, so that solutions can be designed to mimic or induce the protective “memory” of good glycemic control and to inhibit or reverse the sensitizing “memory” of poor glycemic control.

**Behavioral Interventions Improve Metabolic Control:** In combination with good clinical care, psychosocial interventions can improve glycemic control, leading to prevention of diabetes complications. Behavioral research has successfully tested educational strategies, coping skills training, diabetes-related stress management interventions, and behavioral counseling for patients, their families, and significant others. Children can be intensively managed without increasing hypoglycemia; family-focused intervention can yield beneficial glycemic control outcomes, particularly among patients in the poorer range of control before treatment. Research has also shown that self-care autonomy in children with diabetes is associated with adverse outcomes. To counter this, developmentally appropriate parental involvement in diabetes tasks is essential for improved metabolic functioning. There is mounting evidence that adolescents struggling with diabetes adaptation continue to have problems with control as they get older, including a higher likelihood of depression, earlier complications, and disengagement from the health care system upon graduating from pediatric care. These data suggest the importance of focusing on interventions upon diagnosis, leading to metabolic control and preserved psychosocial function. Strategies to help families manage conflict have been developed, and the goal is to find ways to broadly implement them.
The prevention and reduction of complications will be greatly facilitated by the discovery and development of agents that prevent or reverse the cellular and tissue injury induced by type 1 diabetes and hyperglycemia. Each research objective in this chapter addresses a critical area necessary to achieve this overarching goal. An understanding of the molecular mechanisms and genetic risk factors underlying diabetic complications may lead to the identification of new molecular targets for drug development. Application of the latest advances in drug development technology to diabetic complications has the potential to greatly decrease drug development time and improve prospects for clinical success. To test promising drug candidates, animal models are needed that more completely mimic the human pathology of diabetic complications. The discovery of biomarkers and surrogate endpoints for the early manifestations of diabetic complications could allow targeted therapies and potentially shorten the duration of clinical testing, thus removing a significant barrier to achieving clinically useful therapeutics for diabetic complications.

**Molecular Mechanisms of Common Pathways in Diabetic Complications**

Understanding of the mechanisms underlying diabetic complications has greatly expanded in recent years as scientists have identified several implicated molecules. Recent technological advances present an important opportunity to fully characterize the disease pathways that cause retinopathy, nephropathy, neuropathy, cardiomyopathy, accelerated atherosclerosis, and other diabetic complications. Basic science discoveries—such as the recent discovery of microRNAs (see Goal VI), which may regulate expression of one-third of all genes—will help researchers studying diabetic complications to identify completely new and unpredicted therapeutic targets and clinically useful biomarkers.

**Research Objective—Identify Molecular Pathways of Hyperglycemia Damage:**

- Identify the mechanisms of vascular proliferation in diabetic retinopathy.
- Discover the mechanisms leading to diabetic neuropathy that can occur through impaired blood vessel function and other causes, such as AGE formation and alterations in nerve growth factor signaling.

The identification of the cellular and molecular pathways involved in diabetic complications provides a strong foundation for research on key regulatory steps in these pathways that should lead to exciting and clinically relevant discoveries. These breakthroughs in basic research on cellular pathways promote interdisciplinary research with investigators in other basic science and disease-based fields. The goals listed above on oxidative stress, inflammation (RAGE), and angiogenesis are central not only to diabetic complications, but are also involved in numerous other diseases, such as cancer and atherosclerosis. Therefore, research in this area will benefit from and contribute to the much broader biomedical research endeavor.

**Research Objective—Clarify Mechanisms Linking Fuel Utilization and Heart Disease:**

- Characterize the factors controlling increased fatty acid accumulation and mitochondrial oxidation in the development of diabetic cardiomyopathy and accelerated atherosclerosis, as well as the mechanisms by which this cellular lipotoxicity induces cell damage.

The heart has extraordinarily high energy requirements related to its function as a pump throughout life. The energy demands of the heart are met through a high-capacity mitochondrial system that is well suited to oxidize fatty acids and glucose to generate energy. In the insulin-deficient state, this high-capacity mitochondrial system is pushed to the limit through increased reliance on fatty acid oxidation as the energy source. Whereas the increase in cellular fatty acid utilization in the insulin-deficient state is initially an adaptive response, evidence is emerging that this increased fatty acid import and oxidative flux lead to deleterious consequences relevant to the pathogenesis of myocardial and vascular disease. Early studies in this area have identified a number of potential mechanisms linking increased fat utilization to cellular toxicity, including the accumulation of lipid products that could trigger signaling events leading to apoptosis (cell suicide); generation of ROS via increased oxidative flux through mitochondria and peroxisomes; and secondary damage to mitochondria, leading to bioenergetic abnormalities. Future studies related to each of these potential...
mechanisms will be important for enhancing understanding of the pathogenesis of cardiovascular toxicity in type 1 diabetes. Moreover, identification of relevant cellular events involved in this response could pave the way for identification of new therapeutic targets and biomarkers.

Research Objective—Understand the Systems Biology of Diabetic Complications:

- Apply a systems biology approach to research on diabetic complications.

The pathogenesis of diabetic complications encompasses much more than the cellular responses to the metabolic defects of diabetes. Each known diabetes-induced abnormality within a cell can be thought of as connected in a circuit-like arrangement with other intracellular molecules. Similarly, the pathology in one cell type is also connected in a circuit-like arrangement with other cell types in a specific tissue, and each tissue type is likewise connected to other tissues and organ systems, with changes in one nodal component influencing many other points in the network. The emerging field of systems biology will have a major impact on progress in this area. Systems biology is a powerful, mathematically based discipline that seeks to analyze the many simultaneously occurring changes in intracellular, intercellular, and inter-organ contexts as complex, interconnected circuits that have nodal control points, much like the electronic circuits on a microchip. The use of a systems biologic approach can lead to models of in vitro and ex vivo systems, both of which would be useful for identifying mechanisms of injury and testing targets for therapy.

Metabolic Memory

The phenomenon of hyperglycemic memory presents a paradox: Patients in the DCCT with long-term exposure to a higher level of hyperglycemia remained more susceptible to complications, even with subsequent lower levels of hyperglycemia. In contrast, lower levels of hyperglycemia made patients more resistant to damage from subsequent higher levels. How can a finite period of different degrees of hyperglycemia result in different susceptibilities to complications? The discovery of the molecular and cellular basis of both types of metabolic memory is urgently needed so that solutions can be designed to prevent or reverse the damaging “memory” of high hyperglycemia, and to mimic or induce the protective “memory” of lower levels of hyperglycemia. Unlike the pathogenesis of diabetic complications, the molecular mechanisms underlying metabolic memory are virtually unexplored.

Goal V: Prevent or Reduce the Complications of Type 1 Diabetes

Research Objective—Discover the Molecular Mechanisms of Metabolic Memory:

- Study epigenetic factors involved in metabolic memory.

The Director of the National Human Genome Research Institute recently noted that there is an emerging recognition that scientists must move beyond their longstanding focus on the inherited “spelling” of people’s DNA code and the occasional mutation or outright “misspelling.” He noted that epigenetic changes do not alter genetic spellings, but may account for many cases of cancer and other diseases.

Human cells have tens of thousands of genes, each with its own job, such as producing energy or overseeing cell division. But only certain genes are active at any given time or in a given cell type, while the rest are appropriately dormant—a grand orchestration that adds up to a smooth-running life. This orchestration is determined by environmentally induced changes in molecules that coat the DNA. It has long been known that even identical twins have minor physical variations and differences in characteristics, such as susceptibility to disease. Recently, two dominant epigenetic changes were studied in identical twins: (1) DNA methylation, in which enzymes inside a cell attach a minuscule molecular decoration to a gene, deactivating that gene; and (2) histone acetylation, in which a dormant gene is made active again by the attached chemical group. These altered genetic settings can last a lifetime, and could be important for diabetic complications, if hyperglycemia can lead to these permanent genetic alternations. For example, a period of hyperglycemia could irreversibly turn off a gene that protects against diabetic complications. The ability of hyperglycemia to elicit epigenetic changes may be associated with different stages of development. Therefore, it could lead to treatment strategies that would promote intensive therapies during critical windows of development.

- Investigate the role of mitochondria in metabolic memory.

Much progress has been made in understanding the complex biology of mitochondria, which are the major source of hyperglycemia-induced ROS. Scientists now recognize that mitochondria are not all the same, but rather have important functional differences. Furthermore, mitochondria are not static structures in the cell. Rather, they continuously fuse to form larger organelles or pull apart to form smaller organelles. The processes underlying these changes are beginning to be understood, but aberrations induced by diabetes and different degrees of hyperglycemia are important new areas that will likely yield new insights into hyperglycemic memory.
Understand the regulation of the antioxidant response element.

Perhaps not surprisingly, cells have their own protective antioxidant machinery. In a nematode model organism, cells responded to oxidative stress by activating a previously sequestered transcription factor (called Nrf2 in humans), which controls the expression of a diverse set of genes involved in decreasing ROS in the cell. An important research focus is the identification and regulation of proteins in this pathway, including proteins that bind to a special promoter element called the antioxidant response element (ARE) after activation by ROS. Such research will be critical to advancing understanding of hyperglycemic memory.

Genetic Factors

As with all complex diseases, the occurrence and progression of diabetic complications vary markedly among patients. Some patients have type 1 diabetes for over 50 years with minimal complications, while others manifest severe disease or death within 15 years after diagnosis. The control of blood glucose, as well as blood pressure and blood lipid profiles, are important factors in predicting the risk of complications, but they only partially explain the risk of complications for an individual patient. Therefore, genetic factors have been investigated for their influence on the risk of developing complications. An understanding of the genes involved in the susceptibility to or protection from diabetic complications can lead to both a better understanding of the pathophysiologic mechanisms, as well as new biomarkers and molecular targets for drug development.

Research Objective—Identify Genes Conferring Susceptibility and Resistance to Diabetic Complications:

Discover genetic modifiers for diabetic complications.

As genes are identified that impact susceptibility to diabetic complications, a new area of research has emerged that will make it possible to identify genetic modifiers of the clinical manifestation of complications. With the completion of the genetic map known as the International HapMap Project, and new high-throughput genotyping technologies, this promising area of research holds great potential for understanding genetic determinants of the varying clinical severity of diabetic complications. These modifying genes are genetic variants that are distinct from disease susceptibility genes and that modify the phenotypic and clinical expression of the disease genes. Studies show that genetic modifiers can be “tipping point” genes. This term means that one gene changes the whole phenotype in an all-or-nothing fashion, much like switching a power switch “on” or “off.” This paradigm contrasts with the incremental effects seen with changes in a large number of nonmodifier genes. Many examples of modifier genes are known in humans and model organisms. In fact, the most general lesson learned from experiments with genetically engineered mice may be the profound influence of genetic background on the phenotypic consequences of the engineered variant. Early studies suggest that genetic variants with modifier effects are probably also common and diverse in humans.

Current strategies to meet this objective involve the following three ongoing research consortia, which are addressing the genetic factors that either predispose patients with diabetes to or protect them from developing complications: (1) Genetics of Kidneys in Diabetes Study (GoKinD); (2) Family Investigation of Nephropathy and Diabetes (FIND); and (3) Epidemiology of Diabetes Interventions and Complications Study (EDIC). These consortia have collected a large number of samples from patients and families with and without diabetic complications, which they plan to release to interested investigators. One important strategy to validate findings from the human studies will be through the use of animal models. Candidate genes could be tested for their effects in animals through the use of the Animal Models of Diabetic Complications Consortium (AMDCC) and the Mouse Metabolic Phenotyping Centers (MMPC).

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Because complications are likely to result not only from hyperglycemia, but also from a susceptibility to later pathophysiologic steps, such as inflammation or aberrant angiogenesis, a number of modifier genes may be relevant to diabetic complications. Discovery of these modifier genes will require integrated studies of different strains of mice, and comparisons of their genetic similarities and differences.
Accomplishing this research objective will also require careful characterization of patients with type 1 diabetes who have increased susceptibility or resistance to diabetic complications.

**Animal Models**

Animal models that mimic the human development of diabetic complications are desperately needed for research on mechanisms and for drug development. An essential step in developing new therapeutics is to test the efficacy of these agents in animals. Despite the remarkable genetic and physiologic similarities between humans and animal models in both health and disease, many other properties are unique to humans. This limitation means that animal models, while enormously informative, are only indirectly relevant to human disease. One of the reasons is that, in comparisons of mouse versus human genes, “similar” is not “the same.” This distinction means that even subtle differences in gene sequence can lead to functionally important differences in suppression or enhancement of the phenotype by non-conserved amino acids, and responses in mice to potential human therapeutic agents may be very misleading.

**Research Objective—Develop More Human-like Animal Models of Diabetic Complications:**

- Develop human-like mouse models for diabetic complications.

A major opportunity in this field stems from cancer research, where mouse models with greater fidelity to human disease are made by substituting critical human genes for the mouse equivalent. Fortunately, significant advances have been made in the genetic modification of animals, so studies to replace rodent genes with human or human-like genes are feasible.

Engineered animal models of diabetic complications with human versions of genes will have more direct relevance to questions about complications pathogenesis and, equally important, will have a much greater accuracy in predicting which novel therapies will most likely work in humans. The AMDCC has already produced several new mouse models of diabetic heart, vascular, and kidney disease, and is organized to create better mouse models with relevant human and human-like genes. The MMPCs provide standardized, high-quality metabolic and physiologic phenotyping services for mouse models of diabetic complications.

- Utilize large animal models of diabetic complications.

In addition to mice engineered for relevance to human disease, large animal models that more closely resemble human physiology and disease development will also be needed to accelerate the process of “bench-to-bedside” research in the search for new, effective therapies for diabetic complications. For example, inducing diabetes in pigs with the drug streptozotocin provides a model of atherosclerosis that is relevant to this complication of human type 1 diabetes. Validation in such large animal models of potential therapies found effective in mouse models would greatly help to narrow the field of compounds for treating complications that are most likely to succeed in human trials.

Furthermore, large animals may be useful models of complications for which mice are physically too small. For example, reduced nerve blood flow is currently one of the most popular hypotheses for the generation of nerve damage in diabetes, but it has not yet been possible to measure this in mice, whereas larger rodents are widely used for such studies.

**Biomarkers and Surrogate Endpoints To Facilitate Clinical Trials**

The multi-organ damage caused by type 1 diabetes progresses silently for many years before presenting clinically with the signs or symptoms of disease. It then takes many more years before the occurrence of a well-defined event, such as a heart attack or kidney failure. Therefore, detection of early damage to cells and tissue by newly discovered biomarkers is critical for risk stratification of patients. In addition, as tissue damage progresses, the pathophysiologic mechanisms involved in progression are likely to include many more complex elements than are involved in the early initiation phase. This complexity makes therapeutic development more difficult and reversibility of the damage less likely. However, exciting results from islet transplantation trials have provided evidence that some complications can be reversed after many years of normal glucose levels.

Biomarkers include the results of a variety of procedures, including laboratory tests, biopsies, clinical testing, and diagnostic images. Development and validation of biomarkers occur over several phases, from discovery of molecular targets or development of new technologies, to testing with patients and controls, to validating results in clinical trials. Examples currently in clinical use include excretion of small amounts of protein in the urine as a biomarker for diabetic kidney disease, exercise echocardiograms as a clinical test for heart disease, and intravascular ultrasound as a diagnostic imaging technique for evaluating atherosclerosis.
Research Objective—Identify Biomarkers or a Combination of Biomarkers for Earlier Detection of Cell and Tissue Damage:

- **Validate newly developed biomarkers.**
  Newly developed biomarkers that need further evaluation include: (1) measurement of intraepidermal nerve fiber density in small skin biopsies as a biomarker of diabetic peripheral neuropathy; and (2) images obtained from noninvasive magnetic resonance imaging (MRI) techniques as a biomarker of diabetic coronary artery disease. Additionally, the development of functional and qualitative assays of endothelial progenitor cells may be useful as biomarkers for cardiovascular risk.

- **Discover specific molecular targets and innovative technologies for early biomarker development.**
  Research is urgently needed to optimize measurements of known molecular pathologies, such as ROS production and RAGE expression in vivo. Discovery of new biomarkers will encompass signature patterns of gene expression (genomics) or protein expression (proteomics). In addition, integration of these approaches with novel, noninvasive imaging techniques holds particular promise for evaluating metabolic and pathologic changes over time. For example, magneto-fluorescent, multimodal nanoparticles have been successfully targeted to activated vascular endothelial cells in vivo using phage display-derived peptide sequences.

Collaborations among investigators having expertise in complex imaging technologies with investigators having expertise in the molecular cell biology of diabetic complications are likely to produce major advances in this field. These advances will then need to be validated in large clinical trials.

Research Objective—Validate SurrogateEndpoints for Assessing the Progression of Complications in Clinical Trials:

- **Develop surrogate endpoints for clinical trials in diabetic complications.**
  At present, the development of therapeutics for diabetic complications is severely constrained because the slow progression rate of complications requires clinical trials of long duration in order to detect changes in outcomes. Surrogate endpoints are biomarkers that are strongly associated with and predictive of disease outcomes. Valid surrogate endpoints can measure the potential of new therapeutics, as well as be used to provide a strong scientific rationale for longer clinical trials and their prioritization. They would decrease the risk and improve the planning of clinical trials, and thereby encourage development of therapeutics for the complications of diabetes. Developing surrogate endpoints for diabetic complications is an important goal for all the groups involved in drug development, and collaborations among these groups will speed validation and acceptance of new endpoints.

Therapies To Improve Patient Health

Hyperglycemia and the other metabolic effects of type 1 diabetes cause cell and tissue changes that have the potential to be prevented or reversed by treatment. Beyond the vascular diabetes complications, there are psychosocial morbidities associated with the chronic disease that impair quality of life and limit the ability to optimally manage diabetes. Thus, researchers should pursue an array of strategies to prevent or reverse diabetic complications and improve patients’ quality of life. Therapeutic approaches range from finding and testing agents to selectively modify molecular targets responsible for diabetic complications, to combining behavioral and technological approaches to improve patient management and glycemic control.

Research Objective—Identify Therapeutics That Prevent or Reverse the Development and Progression of Diabetic Complications:

- **Use high-throughput screening of molecular libraries to find new therapeutics for diabetic complications.**
  The great success of new drug development over the past 40 years was based on using naturally occurring molecules as leads to design close analogues and derivatives. Classical bioassays and biochemistry were used to select compounds that competed with the native molecule for the same active site. Over the past decade, however, this model has been supplanted in large part by a new strategy. This new strategy involves the automated synthesis of large numbers of molecules (called a library) through a process called combinatorial chemistry, followed by screening in rapid biological assays in a process called high-throughput screening (HTS). A demonstration project that is screening a library of FDA-approved drugs in assays for diabetic complications is currently under way. In addition, it will be essential to outline and have available critical follow-up mechanisms to assist investigators in developing lead candidate molecules in the process that leads to clinical trials.

- **Improve the high-throughput assays for diabetic complications.**
  HTS has been tried for diabetic complications, but the
general consensus is that the HTS assays used were a poor simulation of the in vivo processes involved in diabetic complications. To find effective new drugs, it is essential to create and optimize cell- and simple organism-based models of the processes involved in determining the initiation, progression, and regression of diabetic complications. These assays can also be used to test libraries of existing drugs to determine their effectiveness in pathways relevant to diabetic complications. Several of the major research advances described earlier in this chapter have identified specific molecular targets or pathways that need to be pursued as targets for drug development. Other areas still need targets to be identified. One important example is the search for a molecular target that determines the regenerative capacity of diabetic blood vessels and other structures damaged by diabetic complications.

- **Apply the latest advances in drug development technology to diabetic complications.**

Through use of the combinatorial chemistry-HTS approach alone, fewer than expected viable drug candidates have reached the stage of clinical trials. More recently, a complementary strategy to HTS has emerged. This process uses computer-based virtual screening, multidimensional compound property optimization, and de novo design of drug-like molecules, which make it possible to identify not just active compounds, but compounds with high potential for optimization into drug-like lead series. While later stages of drug development require the substantial financial resources of pharmaceutical and biotechnology companies, the highly innovative NIH Molecular Libraries Initiative (MLI), a component of the NIH Roadmap for Medical Research, complements the private sector drug discovery effort by creating and screening a broader range of compounds, and assaying their effects on a broader range of targets. A significant expansion of the MLI into the area of diabetic complications could link innovative and creative academic researchers with the tools and expertise of small molecule discovery and development. This approach would decrease the time required for drug development and greatly improve prospects for clinical success. Mechanisms to enhance interaction between diabetes investigators and scientists leading the NIH Roadmap initiatives would also help facilitate more rapid development of candidate molecules for testing in type 1 diabetes.

- **Encourage the translation to human application of promising new therapies.**

There are a number of critical steps in the translational process for developing new therapeutic agents. Critical for fulfillment of the promise of HTS is that all promising leads undergo careful testing using the best available animal models of diabetes complications. These tests need to be conducted rigorously and in parallel with validated and consistent outcome measures. The Diabetic Retinopathy Clinical Research Network (DRCR.net) has been a successful model using private and academic clinical practices to test new therapies for diabetic retinopathy. Similar clinical networks for complications, such as nephropathy, neuropathy, wound healing, or cardiovascular disease, may help propel the translation of therapies to the clinic. Animal studies are a critical prelude to testing the most promising new therapeutic agents in human patients. Given the long timeframe necessary to definitively validate new therapeutic agents in humans, strategies to assess the promise of new agents in early phases of clinical testing are critical. This early clinical testing will likely require the use of a panel of existing and new markers for human disease.

**Research Objective—Mitigate Psychosocial Complications and Comorbidities of Diabetes To Improve Quality of Life:**

- **Clarify the bidirectional influences of depression as a complication and potentially modifiable risk factor for type 1 diabetes complications.**

Depression commonly occurs in patients with type 1 diabetes. In addition to depression in patients with type 1 diabetes, high rates of depression are observed in their parents and may have serious consequences for family quality of life and diabetes management. Depression in patients with type 1 diabetes is associated with hyperglycemia, increased insulin resistance, and increased risk of complications, particularly coronary heart disease. These observations are not entirely explained by the adverse effects of depression on behavior (e.g., decreased adherence to medical therapy, smoking, inactivity, obesity) and may reflect other psychophysiologic factors. Research is needed to determine whether such factors exist, and how they may adversely affect the course of diabetes. Conventional antidepressant treatments are effective in the presence of diabetes, with depression improvement leading to significant reductions in HbA1c levels. Some antidepressant drugs may have beneficial effects on diabetic neuropathy. Clinical studies could compare pharmacologic and nonpharmacologic management approaches, singly and together, in primary and secondary prevention of depression in type 1 diabetes, to evaluate the effects of antidepressant treatments on diabetic complication outcomes.
Research Objective—Combine New Technology for Diabetes Management with Behavioral and Translational Research:

- **Design family-based interventions to improve patient management of diabetes.**

The diagnosis of type 1 diabetes can plunge a family into a long-term crisis mode with parents overwhelmed by the associated burdens and fears. Diabetes-related family conflict is a key factor limiting diabetes control. Children and families who show psychosocial adjustment difficulties early after diagnosis are at risk for poor long-term behavioral adaptation to diabetes. Research is necessary to develop behavioral approaches that improve family function and, ultimately, metabolic control in the patient. This will involve studying how patients and their families perceive information, adapt to new technology, and think about risk, in order to learn what motivates them to initiate and maintain behavioral change.

- **Identify strategies to improve adherence to therapy in adolescents and young adults with type 1 diabetes.**

The concept of metabolic memory (described previously) argues for promoting good glucose control as early as possible after diagnosis. However, it has been documented that adolescent years are characterized by lower adherence and elevated HbA1c levels. There is strong evidence that insulin resistance of puberty, the changing nature of the parent-child relationship, and the normal developmental tasks of autonomy, identity formation, and peer affiliation all contribute to suboptimal diabetic control in adolescence. Furthermore, empirical evidence has documented that loss to medical follow-up is a strong predictor of later complications, and the young adult period (18-30 years of age) is a vulnerable time for erratic medical care. Controlled studies have identified a range of strategies for improving glycemic control during the early adolescent years—for example, reducing family conflict over diabetes management, improving parental support for diabetes management, and negotiating realistic expectations for adolescent behavior and blood glucose levels. However, investigations are needed to determine the most effective ways to translate these strategies into routine pediatric care for adolescents with type 1 diabetes and their families.