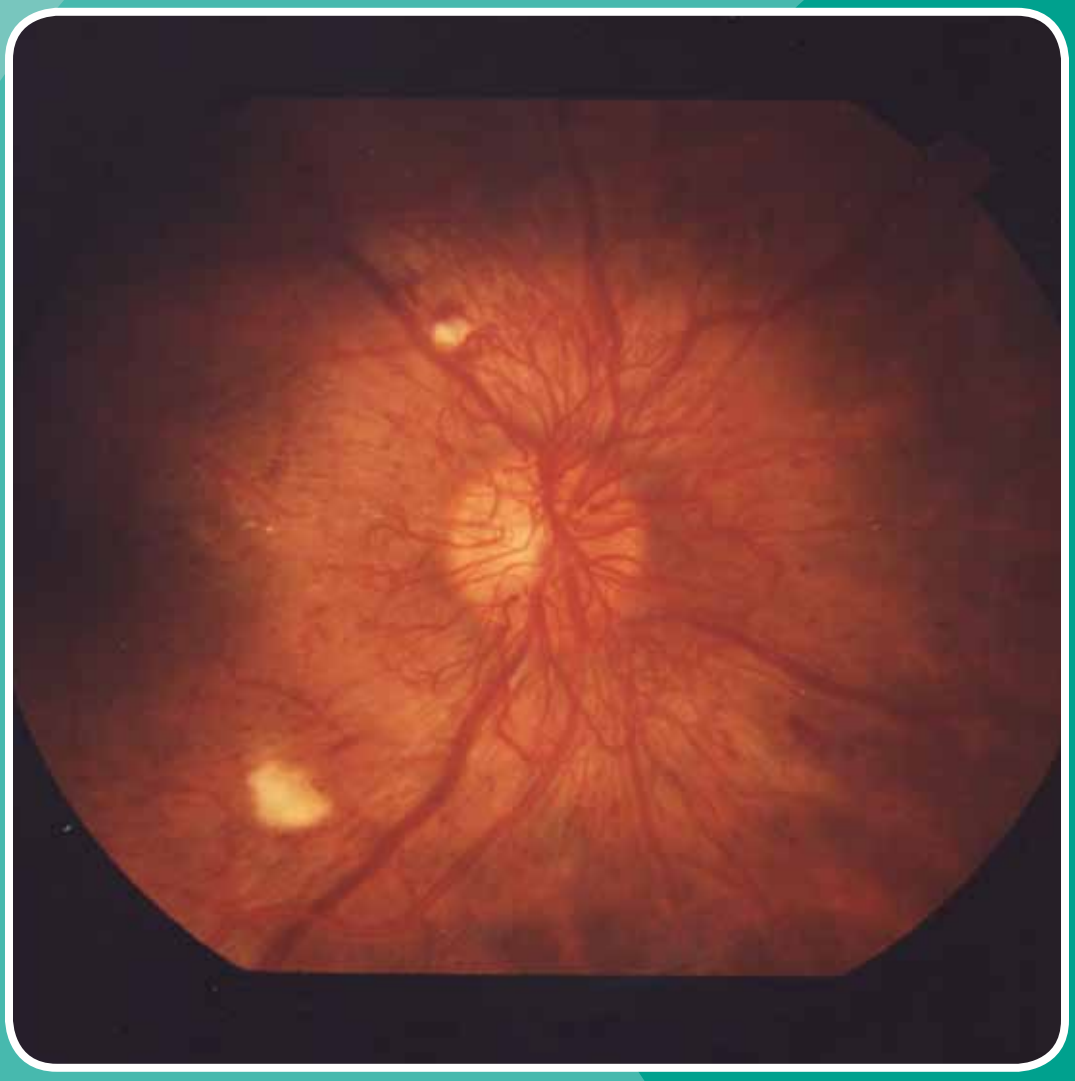


Diabetic retinopathy, damage to the tissue that lines the back of the inside of the eyeball, is the most common form of diabetic eye disease and is a serious complication of diabetes. In retinopathy, the small blood vessels that supply the retina with oxygen and nutrients proliferate and are weak and easily damaged. As a consequence, sight is impaired. This photograph shows a retina exhibiting retinopathy. Research on blood vessel proliferation has led to a promising new treatment to reduce its effects in the eye. (*Photo credit: National Eye Institute, NIH.*)

DIABETES COMPLICATIONS

contents:

INTRODUCTION

Diabetes kills with neither speed nor precision, but with stealth and the slow accumulation of insults. It can rob a person of the ability to see, feel, think, walk, and have sex. The cost of diabetes to an individual cannot be calculated, but for the United States an estimated $58 billion was spent in 2007 on care for chronic diabetes-related complications (1). Damage to the large blood vessels (macrovasculature) causes accelerated atherosclerosis and puts people with diabetes at a 2- to 4-fold higher risk of dying from heart attack or stroke than individuals of the same age without diabetes (1). Damage to smaller blood vessels (microvasculature) results in end-organ diseases that significantly erode quality and length of life. For example, diabetic eye disease, or retinopathy, is the most common cause of vision loss in working age Americans (1). Similarly, diabetic kidney disease, or nephropathy, is the most common cause of kidney failure, which can only be treated with dialysis or kidney transplantation and is associated with a dramatic increase in mortality post renal failure, especially from cardiovascular disease. Diabetes not only accelerates coronary artery disease but also damages the small blood vessels within the heart, as well as heart muscle cells (cardiomyocytes), leading to diabetic cardiomyopathy, which markedly increases the risk of heart failure. Diabetes-induced nerve damage, or neuropathy, can cause pain, loss of sensation, incontinence, impaired gastric motility, and sexual dysfunction. A combination of blood vessel and nerve damage contributes to poorly healing foot ulcers that result in over 65,000 lower limb amputations per year (1). People with diabetes are also at increased risk for periodontal disease, pregnancy-related complications, bone fractures, depression, Alzheimer's disease, and other conditions, including higher rates of cancer and infections.

A person's risk for complications is influenced by the duration and management of diabetes, genetics, and the presence of other risk factors and health conditions. Thus, multiple medical strategies are needed to prevent complications or slow their progression. Landmark clinical trials, such as the NIH-supported Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), have demonstrated that intensive daily management to control blood glucose levels early in the course of diabetes can reduce the risk of developing macrovascular and microvascular complications for people with either type 1 or type 2 diabetes. Multiple, rigorous clinical trials have proven that treatment to lower blood pressure and cholesterol levels reduces cardiovascular disease risk and that direct interventions, such as revascularization, can improve cardiac outcomes. Blood pressure control also reduces the risk of retinopathy and nephropathy. Regular eye exams and timely laser therapy to treat diabetic retinopathy reduce the development of severe vision loss. All these evidence-based strategies reduce the occurrence of complications and improve the quality of life for people with diabetes. However, while representing important progress in diabetes care, they also have important limitations. Most significantly, current therapies reduce but do not eliminate the risk of complications and do not directly prevent the cellular damage induced by diabetes—i.e., they do not address the root cause of the damage. In addition, optimal diabetes care is difficult to achieve and sustain over long periods of time even for highly

motivated individuals. For example, intensive insulin therapy to control blood glucose levels requires extensive efforts by affected persons and also carries its own risks, with an estimated 2 to 4 percent of people with type 1 diabetes dying from the acute consequences of very low glucose levels (severe hypoglycemia) (13). As a result, many people cannot achieve target glycemic goals because their immediate risk of hypoglycemia must be balanced against future long-term complications.

Artery occluded by lipid buildup. Cardiovascular disease (CVD) accounts for two-thirds of deaths in diabetes (1); moreover, CVD rates are as much as 10-fold higher in people with type 1 diabetes than in the general population. *(Image courtesy of National Heart, Lung, and Blood Institute, NIH.)*

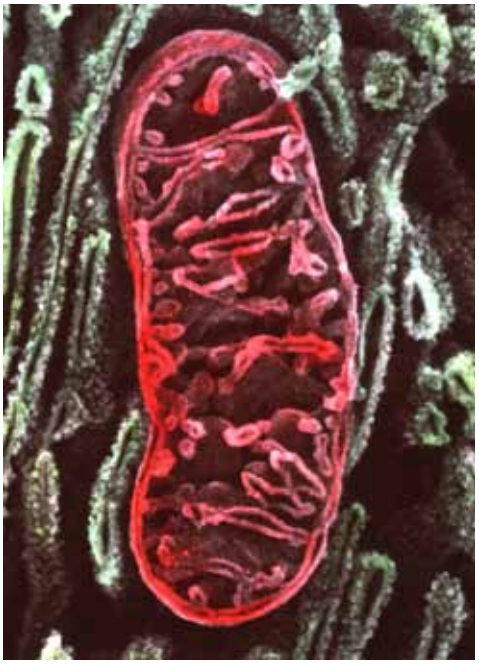
Progress is being made through NIH-funded research at identifying cellular pathways that are affected by diabetes and understanding the interactions among the pathways and their tissue-specific features. This knowledge is critical for developing new treatment strategies and drugs to prevent or treat diabetes complications. Diabetes not only directly damages cells and tissues, but also modifies their repair and regeneration. An appreciation is emerging that a "memory" of past glycemic levels is retained in cells and tissues that affects the course of complications. New tools will allow better study of diabetes complications in humans through large-scale genetic studies, bio-specimen analysis, and imaging techniques. The complexity of complications challenges the research community to form collaborations and apply technological advances to discover therapies that will lighten the burden of diabetes for people living with this disease. This chapter describes both major research advances and new and emerging opportunities for research in a broad array of fields that will make achieving this goal a reality.

RECENT RESEARCH ADVANCES

Scientists working in many disciplines have learned a great deal in the last decade about the molecular underpinnings of diabetes complications, and about clinical approaches to delay onset or progression of these serious health conditions. The following are some major examples of research that has advanced understanding and treatment of diabetes complications.

Multiple Biochemical Pathways Converge To Cause Diabetes Complications: Research has led to the formulation of a new, unifying hypothesis for the major biochemical pathways that contribute to glucose-induced damage of vascular cells: elevated flux through the polyol and hexosamine pathways, accumulation of advanced glycation end products (AGE) and activation of protein kinase C. The hypothesis posits that all of these pathways share the common feature of overproduction of reactive oxygen species (ROS) in the mitochondria (cellular organelles that utilize oxygen to generate ATP, the cell's energy currency), which in turn leads to oxidative stress and cellular damage. Researchers can now use this hypothesis to explore common therapeutic targets for vascular complications of diabetes.

Mitochondrial Dysfunction from Hyperglycemia Correlates with Diabetic Complications: Mitochondria generate energy from glucose and lipids. Researchers have found that mitochondrial function is disrupted by long-term exposure to high glucose levels in ways that are linked to diabetic complications. For example, high glucose levels cause mitochondria to produce more ROS. Inhibition of this mitochondrial oxidative stress in diabetic animal models prevents complications. In people with diabetes, decreased cardiac efficiency is associated with mitochondrial oxidative stress, suggesting that this pathway would be an important target for drug development.



Scanning electron micrograph of a mitochondrion. Human cells contain many mitochondria, which are the "powerhouses" of the cell. Overproduction of reactive oxygen species by mitochondria is suspected to play a role in diabetes complications and is a potential target for therapeutic intervention. (*Image credit: Professors Pietro M. Motta and Tomonori Naguro / copyright ©Photo Researchers, Inc.*)

Inflammation Fuels the Destructive Synergy Between Metabolic and Cardiovascular Disease: Inflammation is now recognized as both a significant contributor to the development of diabetes and a consequence of the diabetic state. Inflammatory molecules, including adipokines, toll-like receptors,

adhesion molecules, chemokines, and cytokines, are increased in both diabetes and the metabolic syndrome and are a driving force in atherosclerosis and other complications of diabetes. A major advance in the field is the identification of the receptor for advanced glycation end products (RAGE), a molecule that bridges the innate and adaptive immune system and that was originally identified as a receptor for proteins modified by AGE. RAGE expression is increased by diabetes, and the binding of RAGE to AGE and a number of endogenous ligands activates pro-inflammatory signaling pathways. Pharmacological blockade of RAGE or genetic deletion of RAGE in animal models is protective against the development of macro- and microvascular complications. In people with type 2 diabetes, blocking RAGE activity is being tested in Phase II clinical trials. Thus, these new findings about molecules important in inflammation could lead to new therapeutic approaches to reduce development of diabetes complications.

The Repair and Regeneration Process Is Impaired in Diabetes: Maladaptive diabetic vasculature causes significant morbidity, as typified by the chronic, non-healing foot ulcer and the poor recovery from impaired blood supply to the heart, brain, and/or limbs. Repair of tissue and revascularization after ischemia occurs in stages that involve cells and molecules in the circulation and wound site. Circulating endothelial progenitor cells (EPC) that contribute to new blood vessel formation were first described in 1997. Several recent studies report impairments in EPC number and function, as well as dysfunction of other stem cell populations, in diabetes. Trials are under way to test the ability of local injection of a person's own EPCs to promote vascularization.

Recent studies on wound healing in response to ischemia show that hypoxia-inducible factor (HIF)-1alpha is the critical transcription factor that regulates new blood vessel formation. Metabolic by-products of glucose affect both HIF-1alpha stability and activation, resulting in suppression of HIF-1 alpha target genes. The HIF-1alpha regulated cascade and other molecules involved in the repair process, such as vascular endothelial growth factor (VEGF), Akt1, nitric oxide, and netrin-1, might serve as targets for therapeutic intervention to restore tissue responses to injury.

Long-Term Clinical Trials Revealed the Phenomenon of "Metabolic Memory" in People with Diabetes: In follow-up studies of two large clinical trials on glycemic control in type 1 and 2 diabetes, participants who intensively managed their blood glucose during the trial have maintained a lower risk of complications for more than 15 years, even though after the trial ended, their glucose control gradually became indistinguishable from that of the participants who had received standard glycemic control measures. This apparent long-term benefit of a relatively short period of intensive glucose control has been termed metabolic memory. These results underscore the importance of intensive glucose management from the earliest stages of diabetes and point to the need for research in epigenetics and other potential mechanisms contributing to metabolic memory (see sidebar, "Sustained Effect of Blood Glucose Control on Susceptibility to Complications—'Metabolic Memory'," for more details).

SUSTAINED EFFECT OF BLOOD GLUCOSE CONTROL ON SUSCEPTIBILITY TO COMPLICATIONS—"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 blood glucose control dramatically reduced the occurrence and severity of diabetic microvascular complications—eye, kidney, and nerve disease. After the announcement of the DCCT results, many participants who had been in the standard therapy group adopted more intensive therapeutic regimens, and their level of blood glucose control improved, as measured by the HbA1c test. At the same time, the mean level of HbA1c worsened for participants who had been in the intensive therapy group. The post-DCCT HbA1c values for both groups have become nearly identical during the approximate 15 years of follow-up in the ongoing Epidemiology of Diabetes Interventions and Complications Study (EDIC).

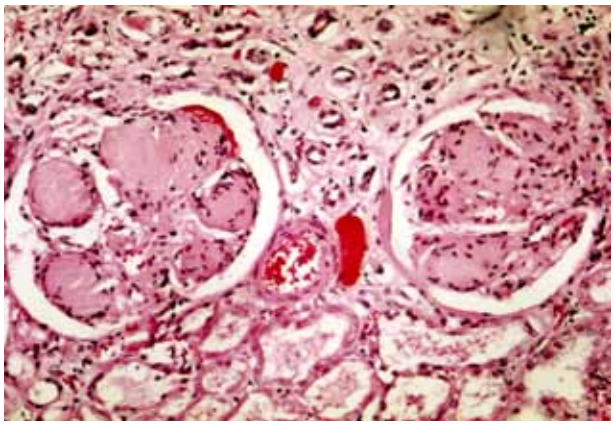
Surprisingly and provocatively, however, the effects of a 6.5-year difference in HbA1c during the DCCT on the incidence of diabetic eye and kidney disease have persisted, and have even become greater over the subsequent years of follow-up. People who had been in the standard therapy group continued to have a higher incidence of complications, even with an improvement in blood glucose control during the EDIC. In contrast, people who had been in the intensive therapy group continued to have a lower incidence of complications, even with a worsening of blood glucose control during EDIC. In addition, early intensive therapy was recently shown to markedly reduce later development of atherosclerotic changes, heart attacks, and strokes. This effect does not appear to be limited to people with type 1 diabetes who have used intensive insulin therapy. The UKPDS and its follow-up study came to the same conclusion for people with type 2 diabetes who used diabetes drugs or insulin to control blood glucose levels: intensive control of blood glucose lowers the risk of complications, and the benefits of intensive glucose control persist over time even if an individual's average glucose levels eventually worsen.

The phenomenon that the level of blood glucose control could have long-lasting effects, called "metabolic memory," elicits a number of questions. How can a finite period of good—or bad—blood glucose control have such long-lasting effects? Is there a point in the development of complications in which the progression becomes relatively independent of blood glucose control? The discovery of the molecular and cellular basis of metabolic memory could suggest therapeutic solutions that mimic or induce the protective "memory" of good blood glucose control and inhibit or reverse the sensitizing "memory" of poor blood glucose control.

Encouragingly, recent studies in cell culture and animal models have shown a phenomenon of metabolic memory similar to the observations in people—thus providing an avenue to explore the basic mechanisms underlying metabolic memory. For example, studies in diabetic dogs and rats have demonstrated that, even after reversing

hyperglycemia, there is a sustained continuation of key microvascular complications and oxidative stress. Other experiments have looked directly at the effect of high glucose on the cells that line the inside of blood vessels, and found that growing these cells in the laboratory for even short periods in high glucose, followed by restoration of normal glucose, causes persistent increases in expression of genes involved in inflammation and signs of oxidative stress. Similar alterations have been seen in samples of vascular cells obtained from diabetic mice, when compared with those from non-diabetic control mice. Key molecular mechanisms and genetic modifications (epigenetic changes) have been implicated in some of these animal and cellular models of metabolic memory, supporting the need for more studies in this area.

Dysfunction of Podocytes and Endothelial Cells Contributes to Diabetic Nephropathy: Diabetic nephropathy is characterized by changes in the glomeruli, structures in the kidney that filter waste products out of the blood for excretion in the urine. Two glomerular cell types—podocytes and glomerular endothelial cells—are implicated in diabetic kidney disease. A reduction in the number and density of podocytes is a strong predictor for progression of diabetic renal disease. A podocyte-specific protein, nephrin, which helps to prevent proteins from leaking into the urine, is decreased in individuals with diabetic nephropathy. In glomerular endothelial cells, a genetic difference that reduces activity of an enzyme, endothelial nitric oxide synthase (eNOS), is associated with accelerated progression of diabetic nephropathy in humans. Diabetes reduces eNOS activity, and diabetic mice lacking eNOS exhibit accelerated and dramatically more severe diabetic nephropathy than wild-type mice. In the future, identifying the cells that are damaged in diabetic nephropathy will help researchers design more sensitive measures of disease progression and find ways of reversing the specific metabolic abnormalities.



Glomeruli, the filtering units of the kidney, are damaged in diabetic nephropathy, impairing normal kidney function. (*Image provided by the Centers for Disease Control and Prevention / Dr. Edwin P. Ewing, Jr.*)

Polyuria Causes Bladder Remodeling: Many people with diabetes develop problems with urination, such as incontinence and incomplete emptying of the bladder. Now, the mechanisms underlying this health issue are starting to emerge. Poorly controlled diabetes leads to increased urine output due to the excretion of the excess glucose. Consequently, the bladder confronts not only the damaging effects of hyperglycemia on the blood vessels and nerves, but also exceptionally high urine volume (polyuria). In experimental models, increased

urine output leads to rapid and substantial bladder hypertrophy and dysfunction that are similar to changes observed in diabetic rats. Those similarities suggest that increased urine production may be a significant factor in the early storage dysfunction of diabetic bladder disease.

Diabetes Increases the Risk of Cognitive Impairment, Alzheimer's Disease, and Depression: The impact of diabetes on the nervous system has been studied largely in the context of understanding peripheral neuropathy. Over the last decade, the earlier onset of type 2 diabetes and refined behavioral, psychological, and neuroimaging techniques has led to an appreciation of central nervous system (CNS) injury that can be separated from other aspects of aging and vascular disease. Diabetes appears to contribute to cognitive impairment during early childhood, when the brain undergoes structural changes and development. Longitudinal studies report lower intelligence quotient (IQ), decreased mental efficiency, and worse school performance in children with type 1 diabetes compared to children without diabetes. Research has also demonstrated that diabetes contributes to cognitive dysfunction during late adulthood, when the brain undergoes neurodegenerative changes due to aging. Older adults with type 2 diabetes show a 1.5- to 2.0-fold increased risk of cognitive decline compared to adults without diabetes. Cognitive deficits in type 2 diabetes are associated with structural brain abnormalities that include cerebral atrophy and lacunar infarcts (a type of stroke). In addition, research studies indicate that individuals with type 2 diabetes have a greater than 2-fold increased risk of developing Alzheimer's disease (AD) compared to individuals without type 2 diabetes. The reduced insulin production in type 1 diabetes and insulin resistance in type 2 diabetes both can generate AD-like pathology in the CNS.

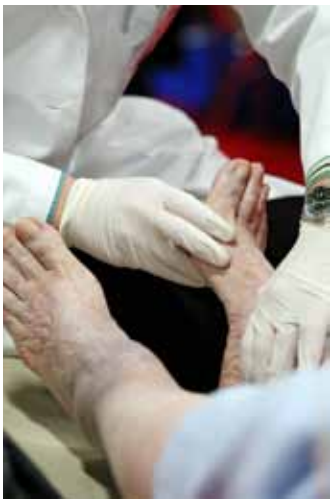
Depression is also associated with both type 1 and type 2 diabetes and this association is bi-directional, with each influencing the presentation of the other. Studies have shown that the comorbidities of diabetes and depression are linked to poor glycemic control, elevated inflammatory markers, microvascular complications, and cardiovascular disease. Therapeutic approaches to CNS diseases will be more successful with a better understanding of the biological mechanisms that link them to diabetes.

Major Clinical Trials Guide Therapy To Reduce Diabetes Complications: In previous decades, large clinical trials proved that substantial reductions in complication rates are possible when HbA1c, blood pressure, and LDL cholesterol are controlled, but important questions remained about the optimal therapeutic targets for these risk factors. Glucose control can reduce the development of microvascular complications in people with either type 1 or type 2 diabetes, but its direct effect on macrovascular complications is less clear, at least in people with long-standing type 2 diabetes. Moreover, hyperglycemia, as measured by HbA1c, accounts for only part of an individual's risk of complications. Hypertension and dyslipidemia are major contributors to diabetes complications, and optimal levels of control of glucose, blood pressure, and lipids remain to be established. Now, a major clinical trial has compared standard versus more aggressive treatment goals for these risk factors in people with long-standing type 2 diabetes and found that current guidelines yielded lower mortality than more aggressive glycemic control aimed at near normal glucose levels. The more aggressive goal of near

normal blood pressure levels yielded no improvement in the primary macrovascular disease outcome. In addition, no benefit in macrovascular outcomes was seen when a fibrate was added to standard statin therapy. These studies of more aggressive therapy exceeding current standards of care do not negate the previously proven benefits of control of glucose, blood pressure, and LDL cholesterol in reducing diabetes complications, but do provide new information helpful in balancing the risks and benefits of therapies and optimizing levels of risk factor control.

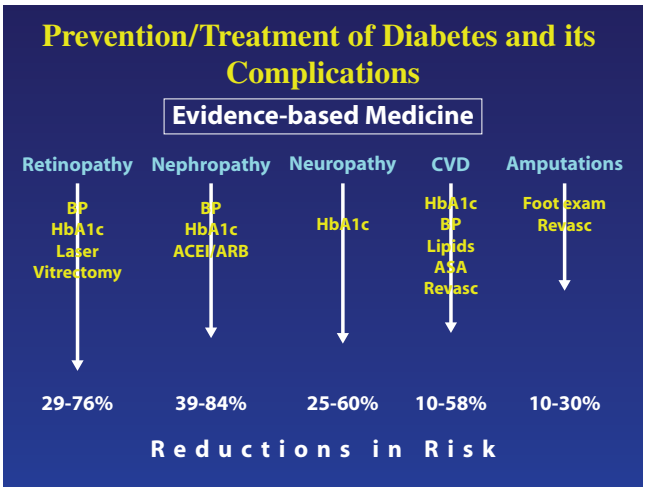
Beyond Hyperglycemia—Other Risk Factors for Diabetes Complications: New data are emerging on risk factors for microvascular complications. For example, the development of diabetic neuropathy is associated with abnormal levels of serum lipids, blood pressure, and urinary albumin, and with obesity. Moreover, emerging clinical and experimental data highlight roles for insulin resistance, increased peripheral insulin levels, and the loss of growth factors (C-peptide, neurotrophic factors, cytokines, etc) in the pathogenesis of diabetic complications. Taken together, these considerations highlight the fundamental requirement for understanding the synergistic pathways that contribute to diabetic complications and then developing combined therapeutic approach strategies tailored to individuals.

Closing in on New Methods for Assessment of Diabetes Complications: Diabetes complications develop over decades. Useful biomarkers, such as HbA1c for glycemic control, are snapshots of the disease process that can predict the future clinical outcomes and reflect the underlying mechanisms. Research in a variety of areas has led to exploration of promising new tools and techniques for assessing complications. Skin autofluorescence shows promise as a noninvasive surrogate marker for AGE formation in the pathogenesis of diabetic complications. Skin intrinsic fluorescence levels can predict cardiovascular morbidity and mortality and kidney disease in people with diabetes. Diabetic peripheral neuropathy has two new tests under development that eventually may enhance the potential for identifying therapies. These tests, skin biopsies and corneal confocal microscopy, assess the structure of distal terminals of sensory neurons and quantify small fiber nerve damage in minimally invasive or noninvasive assays. Biochemical risk factors, vascular imaging, and stress testing can help to define CVD risk in persons with diabetes. Continued efforts to test and develop biomarkers, tests, and other tools to detect diabetes complications will be important for prevention and treatment of these conditions.



Comprehensive foot exam. Peripheral nerve and vascular damage in diabetes interferes with sensation and healing from tissue injury, heightening risk for amputations. Research is moving forward on treatments to control infections and promote or restore healing. *(Photo credit: © iStockphoto.com/jorgeantonio)*

New Therapeutic Approaches for Microvascular Complications: Over the past decade, new therapies have been developed to prevent or treat complications in people with diabetes. For example, a new treatment targeting angiogenesis has shown success in treating diabetic macular edema, an advanced complication of the disease. Results from a randomized, clinical trial conducted by the NIH-supported, multi-center, Diabetic Retinopathy Clinical Research Network have demonstrated that eye injections with an anti-VEGF drug, often in combination with laser treatment, result in better vision for more patients than laser treatment alone—nearly half of the patients receiving the drug showed a substantial improvement in vision, compared to 30 percent receiving only laser treatments. Research has also expanded on the previous findings that inhibitors of angiotensin-converting enzyme (ACE) significantly reduce the progression of diabetic nephropathy, with early results suggesting that ACE inhibitors may also benefit patients with diabetic retinopathy and diabetic neuropathy. A recent clinical trial has shown that fibrates, a medication to lower blood lipids, can also reduce the development of diabetic eye disease. The development of new therapeutic options for microvascular complications, in addition to glucose control, can prevent or postpone disability and greatly improve the quality of life for people with diabetes.



Examples of the extent to which therapies have been proven to reduce eye, kidney, nerve, and heart disease, and amputations. BP, blood pressure control; ASA, aspirin; Revasc, revascularization. *(Image courtesy of Dr. David Nathan, Harvard University/Massachusetts General Hospital.)*

KEY QUESTIONS AND FUTURE DIRECTIONS FOR RESEARCH

The 1999 report of the congressionally-established Diabetes Research Working Group (DRWG), *Conquering Diabetes: A Strategic Plan for the 21st Century*, highlighted the importance of battling micro- and macrovascular complications induced by diabetes and of expanding work to understand and overcome diabetic neuropathy, a perspective that has been reinforced in recent plans for research on type 1 diabetes. Since the publication of the DRWG plan for diabetes research, multiple recommendations—such as to expand clinical research and clinical trials for diabetes complications, and to foster multidisciplinary research in these areas—have led to new discoveries and therapeutic improvements. The problems are far from solved, however, and additional research on understanding and preventing complications must not be allowed to lag because diabetes is still a major cause of blindness, renal failure, excess CVD, and several other complications. Moreover, the public health problem is increasing as the incidence of diabetes in the United States has also continued to rise during that time, most alarmingly in children and youth—greatly increasing the population at risk for developing and living with diabetes complications long-term. Described below are research questions and promising opportunities that may be pursued in the next decade to reach the goal of meeting the many challenges posed by the health complications of diabetes.

Metabolic, Biochemical, and Signaling Pathways

Essential cellular pathways are disrupted in diabetes, setting the stage for development of complications. An excess of glucose, lipids, and other molecules causes derangements in metabolic pathways and induces buildup of intermediates with toxic effects in and out of the cell. Mitochondria are implicated in many of these pathways because of their role in converting metabolic products to energy. Also, impaired insulin signaling can change the fuel source from glucose to lipids in cardiomyocytes (heart muscle cells) and eventually lead to an excess of intracellular lipid with harmful consequences for the cells. Metabolic pathways relevant to diabetes also control apoptosis and autophagy, forms of cellular "self-killing" and "self-digestion," respectively. The abundance of molecular pathways affected by diabetes presents the challenge of understanding complex interactions among the pathways, but also the opportunity of providing multiple and potentially complementary targets for drug development.

Key Questions

- **How do the identified molecular pathways associated with diabetes interact within the cell and does this vary for different cell types?**
- **Are there undiscovered molecular pathways that contribute to diabetes complications?**
- **What protective pathways are present and how do they interact with other pathways? Do complications arise from an imbalance of maladaptive to adaptive responses?**
- **What are the relative contributions of hyperglycemia versus impaired insulin and other growth factor signaling in the development of diabetes complications?**

- **What is the effect of large dynamic changes in the levels of glucose and other metabolites in comparison to sustained elevations?**
- **Why do cells exposed to the same systemic factors have different pathologies? Why does the apparently global pathogenic mechanism of increased mitochondrial activity have variable consequences in different cell types?**
- **What is the clinical significance of the identified biochemical changes in the cell induced by diabetes?**

Future Directions

➤ **Develop better tools to assess mitochondrial function, transport, number, fission/fusion states, transcription factors, and DNA.**

Genetic mutations in mitochondrial fusion and fission proteins increase ROS production and cause neuropathies similar to diabetic peripheral neuropathy. Characterization of the mitochondrial fusion/fission states can be accomplished with recently developed fluorescence probes, transgenic mice over- and underexpressing fusion/fission proteins, and novel approaches to quantitate and locate mitochondria using confocal microscopy.

➤ **Improve mitochondrial function in tissues in which mitochondrial dysfunction contributes to complications.**

A decline in the oxidative phosphorylation capacity of mitochondria and an uncoupling of energy production are characteristics of diabetic cardiomyopathy. A better understanding of the regulation of these changes in diabetes, including mitochondrial biogenesis and turnover via autophagy (mitophagy), will direct development of drugs that target the mitochondrial abnormalities.

➤ **Develop a better understanding of the immunologic pathways common to type 1 and type 2 diabetes and diabetes complications.**

Recruitment of the innate and adaptive immune responses is increasingly linked to the development of both type 1 and type 2 diabetes. Inflammation, triggered by the actions of immune cells such as T and B lymphocytes, monocytes/macrophages, and dendritic cells, along with the interaction of these immune cells with vascular cells, contributes to the development of diabetes. These various cell types have also been implicated as causative factors for the complications of diabetes. Identifying shared pathways that may reflect, in part, a mechanistic continuum from cause to complication is an important research goal.

➤ **Develop better tools to study glycation and lipoxidation of proteins.**

Hyperglycemia causes extensive glycation of extracellular matrix (ECM) proteins that leads to their cross-linking, accumulation, and altered binding properties for growth factors and circulating stem cells. This sclerotic process contributes to the pathogenesis of complications and the slow reversal of pathology with euglycemia. Methods that prevent these modifications are needed to understand their role in impaired tissue turnover rate and accumulation of glycated proteins. Novel animal models are also needed that duplicate, in the absence of hyperglycemia, the diabetic changes in ECM.

➤ **Determine if modulators of autophagy affect diabetes complications.**

Autophagy may contribute to the progression of complications and the increased risk and poorer prognosis of several forms of cancer associated with diabetes. Autophagy was discovered in yeast as a stress response, but is now linked to human malignancies and known to be regulated by nutrients and pathways relevant to diabetes. The human homologues of yeast autophagy genes and drugs known to affect autophagy are available to test the role of autophagy in diabetes. A better understanding of the regulation of autophagy may elucidate targets to control this process that may be involved in an excess of cell death in certain complications and a deficiency of cell death in malignancies.

➤ **Develop tools and approaches that produce a more global understanding of the cellular effects of diabetes and a more specific understanding of the effects of diabetes on individuals.**

The field of diabetes complications has benefited from the explosion of new genetic, biochemical, and cell biologic techniques. Appropriate systems biology tools are needed to facilitate integration of genotyping information, mRNA expression, microRNA expression, promoter analysis, proteome expression, and metabolome profiles in order to identify key biological processes and their interactions (see also the chapter on "Type 2 Diabetes As a Multi-Dimensional Disease"). In addition to better computational tools, a deeper understanding is needed of the control mechanisms of mRNA and protein expression levels in the diabetic state, such as ubiquitination, sumoylation, DNA methylation, histone modifications, and non-coding RNAs. Ultimately, this knowledge needs to be applied to clinical diabetes through better access and techniques for understanding pathobiology in individuals with diabetes.

Genetics and Epigenetics

Metabolic control alone does not predict an individual's risk for diabetic complications. Family studies suggest that genetic factors play an important role in the predisposition for a specific type of complication and its progression. The chapter on "Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications" highlights research on finding genes that increase the susceptibility to diabetes complications. In addition to more classic genetics, research in this area has expanded to epigenetics and non-coding RNA. Epigenetic marks include modifications to the DNA or chromatin that do not change the underlying DNA sequence and may contribute to metabolic memory, the observation that the level of prior glucose control has persistent effects on the risk of complications (see sidebar, "Sustained Effect of Blood Glucose Control on Susceptibility to Complications – 'Metabolic Memory'"). The elucidation of epigenetic mechanisms has the potential to identify novel therapeutic targets. Small regulatory RNAs, such as microRNAs (miRNAs), are accepted regulators of mammalian cell phenotype, and have been implicated in the regulation of biological functions associated with diabetes pathogenesis, such as metabolism, insulin secretion, and the immune response. Patterns of miRNA in various cells and tissues may provide useful disease biomarkers, while *in vivo* manipulation of specific subsets of small regulatory RNAs might be used for novel therapeutic strategies.

Key Questions

- **What are the genes that predispose or protect people from developing end-stage**

renal disease, diabetic retinopathy, neuropathy, and other diabetes-associated complications?

- **How do candidate genes identified by genome-wide studies contribute to the pathogenesis of diabetic complications?**
- **How do epigenetic mechanisms fit within the context of other known cellular mechanisms for diabetes complications?**
- **Are epigenetic changes in chromatin responsible for metabolic memory? How do they interact with other persistent effects of glucose control, such as glycation and oxidation of long-lived macromolecules?**
- **Is epigenetics the mechanism by which birth weight determines adult susceptibility to diabetes and coronary heart disease?**
- **What is the role of small regulatory RNA, in particular microRNA, in the development of diabetes complications?**

Future Directions

➤ **Identify the key genetic factors predisposing to or protecting from diabetic complications and define the population genetic architecture underlying this risk.**

Genetic predisposition clearly plays an important role in the development of diabetic nephropathy and severe diabetic retinopathy. Genome-wide association (GWA) studies using single nucleotide polymorphisms provide a means for elucidating which genes are associated with the pathogenesis or protective mechanisms for these complications. Large numbers of individuals are required to provide adequate statistical power to identify significant associations in GWA studies (typically 3,000-10,000 people). Genetic studies are particularly valuable when combined with careful phenotyping of people with diabetes to discern if specific complications (nephropathy, retinopathy, or neuropathy) are present and to characterize genetic and environmental interactions. Given the greater risk of kidney disease in African Americans and other minority groups, GWA studies need to be performed in specific at-risk populations (for more information, see the chapters on "Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications" and "Special Needs for Special Populations").

➤ **Test the role of genes identified from GWA studies.**

GWA studies to identify risk or protective genes implicated in diabetes complications are ongoing. Improved cross-talk between investigators identifying genes and those studying cellular and animal models is essential to determine if genes associated with complications actually are implicated in the pathogenesis of complications. Studies in small and large animal models may be required to address these key questions.

➤ **Incorporate new genomic and epigenomic technologies to evaluate diabetic complications.**

Tremendous advances in epigenome, mRNA, microRNA and whole genome profiling have occurred through highly sophisticated technologies such as ChIP-on-chip, ChIP-Seq, and deep sequencing. In addition, specific patterns of chromatin histone marks and DNA methylation have been identified and shown to be associated with gene expression or repression. The challenges to utilizing these advances for diabetes complications include determining how best to apply

them to a disease model and the need for appropriate bioinformatic approaches to decipher the large amount of data. Cross-disciplinary collaborations and specialized centers would be particularly useful in overcoming these barriers.

➤ **Characterize epigenetic changes or patterns of changes that can be used in population studies to probe the questions of metabolic memory.**

Metabolic memory could be a significant challenge to the prevention of diabetes complications, if poor glycemic control early in the course of diabetes seals an individual's fate for complications. Epigenetic changes in chromatin histone methylation and DNA methylation may be not only the fingerprint of past glucose control, but also the finger that touches various cellular processes. Characterization of epigenetic change may provide targets for intervention when the opportunity for metabolic control early in the course of diabetes has passed. This work will be enhanced by good cellular models and by appropriate human material (e.g., clinical data and biological samples) from longitudinal studies to test for epigenetic changes.

➤ **Investigate the changes in microRNA profiles associated with diabetes complications and the downstream effects of identified microRNA.**

MicroRNAs are short ribonucleotides that bind to messenger RNA to modify protein translation or promote RNA degradation. Knowledge of the function and regulation of microRNA is rapidly expanding. They appear to be sensitive to the extracellular environment and could be important regulators of a cell's response to diabetes. Knowledge of microRNA signatures could be translated to therapies based on novel antagomirs, synthetic analogues of microRNA, to interfere with their involvement in diabetes complications.

Tissue and Organ System Injury

Tissue injury in diabetes results from cell damage and death, impaired communication among cells, dysfunction of nerves and blood vessels, and detrimental responses to systemic signals, such as inflammation. The development of the clinical manifestations depends on tissue-specific responses to injury and impairments in repair and regenerative processes. The knowledge base of the pathologic process in different tissues varies considerably, but in all organ systems a better understanding of the mechanisms is needed.

Key Questions

- **How does systemic inflammation from dysregulation of the innate and adaptive immune systems affect specific tissues, such as the periodontium, bone, and endothelium?**
- **What are the mechanisms of injury in specialized cells, such as podocytes, pericytes, Müller cells, and interstitial cells of Cajal?**
- **What distinguishes cardiovascular, kidney, and urologic disease associated with diabetes from non-diabetes related forms of these diseases? Does diabetes accelerate the same pathologic processes or have unique components?**
- **What mechanisms are responsible for the increased mortality in people with diabetes and end-stage renal failure?**
- **Is there a point in the progression of diabetes complications when the pathologic**

process becomes relatively independent of the diabetes-related factors that initiate it? Is there a point when the progression becomes irreversible?

- **What are the pathological and molecular correlates of autonomic neuropathy? What are the biologic mechanisms involved in the bi-directional associations of depression and diabetes and Alzheimer's disease and diabetes?**
- **To what extent does the pain associated with diabetes reflect peripheral tissue injury versus altered CNS processing and perception of pain?**

Future Directions

➤ **Develop *in vitro* models to study vascular complications.**

Vascular dysfunction in diabetes results from hemodynamic, metabolic, and ECM abnormalities. The interactions among these can be explored through *in vitro* models that resemble the *in vivo* situation. For example, a microfluidic bioreactor could be constructed with ECM proteins and channels seeded with vasculogenic cells in which release of growth factors and flow conditions could be tightly controlled and monitored through online imaging. Such a bioreactor might not only foster exploration of dynamic interaction among factors implicated in pathogenesis of complications, but also allow for a systematic evaluation of potential therapeutics, including small molecules and growth factors.

➤ **Establish bio-repositories of human cells and tissues.**

The study of diabetes complications would benefit from the establishment of a bio-repository of well-characterized human samples. However, the desire to study the effect of diabetes on human tissue is tempered with the complexity of individual variation, inaccessibility of many tissues, and uncontrolled and unknowable variables in patient history and sample processing. Some of these factors can be lessened by technological advances in testing and analyzing large numbers of samples. Patterns that could not be seen with small numbers of individuals and end points may emerge with broad platform testing. Coordination of a major effort to obtain clinical samples of tumor and normal tissue from carefully characterized individuals under standardized processing procedures has started in the National Cancer Institute, and a similar effort could be initiated for diabetes complications. Currently, a large amount of material (including DNA, urine, serum, and immortalized cells) is in storage from multiple clinical trials and large-scale genetic studies of people with diabetic complications. These samples and new collections could be analyzed by mechanistic, genomic, epigenomic, and proteomic studies, with similar samples evaluated by multiple platforms simultaneously to facilitate comparisons. (See also the chapter on "Resource and Infrastructure Needs for Diabetes Research.")

➤ **Determine the mediators of dyslipidemia-induced renal and neuronal injury.**

Dyslipidemia is strongly correlated with complications such as nephropathy and neuropathy. Understanding the pathways that mediate the lipid-associated damage to cells could lead to novel treatments for diabetic complications. Study of these complications in a variety

of dyslipidemic animal models will yield important new understanding.

- **Pursue cross-disciplinary research to understand the basic science for neurovascular disease related to diabetes.**

The understanding of diabetic neuropathy, neuro-retinal abnormalities, and cerebral dysfunction has lagged behind that of vascular complications. The vascular and nervous systems are intimately connected and must be studied as a unit to understand the complexity of the interactions at a basic science level. Cross-disciplinary research is essential for progress in these diseases.

- **Understand the mechanisms by which diabetes affects the enteric nervous system and related elements in the gastrointestinal system.**

The impact of diabetes on the gastrointestinal system is multi-factorial and poorly understood. Diabetic gastroenteropathy results from dysfunction of the autonomic nervous system, enteric neurons, smooth muscle cells, and interstitial cells of Cajal, and the communication between these cell types. An inadequate understanding of the enteric nervous system in health, the lack of a critical number of investigators, and limitations in the methodology and in animal models are challenges to be addressed. An existing NIH-funded gastroparesis consortium that includes bio-repositories of DNA and serum and the emergence of a noninvasive method of obtaining human samples of enteric nervous system samples will support exploration of early defects and yield progress in this understudied area.

- **Explore the "temporal theory" of urinary incontinence and diabetic uropathy.**

Complications related to the lower urinary tract affect more than 80 percent of individuals with diabetes and significantly worsen their quality of life. Recent studies in animal models indicate a temporal effect on the development of diabetic bladder disease. The early phase of diabetes leads to urine storage problems from massive bladder remodeling and compensated bladder function. The late phase causes urine voiding problems manifested by decompensated bladder function from myogenic and neurogenic mechanisms. The "temporal theory" provides a scientific framework for understanding the role of polyuria, urine osmolarity, oxidative stress, autonomic neuropathy, and decompensation of the bladder contractile apparatus in the manifestations of diabetic bladder dysfunction. Identification of mechanistic pathways could lead to the development of effective preventive and therapeutic interventions.

- **Incorporate measures of depression, cognitive impairment, brain vascular lesions, and Alzheimer's disease in longitudinal studies of diabetes complications.**

Progress in diabetes complications has come from the bi-directional flow of results between the laboratory and clinic. Findings from epidemiology studies and longitudinal clinical trials can inform and direct laboratory research on biological mechanisms associated with diabetes and both depression and cognitive impairment. A key mechanistic issue in the development

of cognitive impairment in the elderly is distinguishing the role of vascular disease versus the pathology of Alzheimer's disease. Identification of these mechanisms will allow development of preventive therapies that can then be tested in the clinic. The inclusion of currently available, validated measures for depression, sub-clinical depressive symptoms, cognitive impairments, and central vascular lesions in studies of people with diabetes are critical for progress in understanding the CNS complications of diabetes. Such studies will also be enhanced by including measures of potential biological mediators of these associations.

Tissue Repair and Regeneration

Diabetes leads to tissue injury through damage to the blood vessels, ECM, and parenchymal cells. Normally, metabolic and ischemic insults stimulate repair and regeneration. In diabetes, however, these processes are impaired. Examples include non-healing ulcers in the foot, decreased neovascularization in response to inadequate blood flow (ischemia) in the heart, and the proliferation of bleeding-prone vessels in response to regional hypoxia in the retina. Circulating progenitor cells contribute to normal new vessel growth, but their number and function is altered by diabetes. Restoring the health of these cells or infusing *ex vivo*-modified progenitor cells are new therapeutic approaches. Recent advances in cell reprogramming hold great promise for future cell replacement therapies.

Key Questions

- **Can the complications of diabetes be reversed by stimulating formation of normal new vessels and re-growth of nerves? Is this possible despite continued hyperglycemia?**
- **How do the various pathways leading to abnormal vascular proliferation, loss, and permeability contribute to complications in different tissues?**
- **Can restoration of the regulation and oxygen sensing of HIF-1alpha rescue the diabetic impairments in neovasacularization?**
- **How do dysfunctional repair mechanisms contribute to poor recovery from maternal injuries of childbirth and the resultant increased risk of stress incontinence and female pelvic floor disorders?**
- **How are specific populations of stem/progenitor cells affected by diabetes? Are these abnormalities reversible through optimal diabetes treatment or therapies targeted to stem/progenitor cells?**
- **Will new cell reprogramming techniques, such as induced pluripotent stem (iPS) cells, lead to individualized cell therapy?**

Future Directions

➤ **Elucidate the mechanisms underlying the poor revascularization response to ischemia in diabetes.**

In a person without diabetes, inadequate blood flow in the heart, brain, and lower leg initiates a complex response that leads to the recovery of vascular function. Impairment of several pathways and factors such as HIF-1alpha, VEGF, netrin, and nitric oxide are implicated in the poor vascular response seen in diabetes. The relative importance of different pathways may differ among tissues, particularly in the brain. Developing drugs that normalize these pathways in various target tissues is an important therapeutic goal.

➤ **Characterize the impairments in stem and progenitor cell populations.**

Stem and progenitor cells are crucial for repair and regeneration after ischemia and trauma. Diabetes, possibly through metabolic memory, can lead to decreased function and quantity of progenitor cells, and these impairments are likely to play a role in diabetes complications. A barrier to understanding the impairments includes the known heterogeneity within stem cell compartments, specifically mesenchymal stem cells. Protocols are needed for the culture of progenitor cells and their thorough analysis, including genetic, epigenetic, and transcription factor analysis of individual cells and stem cell populations. Stem and progenitor cell dysfunction could become a biomarker if clear metrics are developed that correlate with diabetic complication rates specific to neovascularization and cardiovascular repair and regeneration. *Ex vivo* therapies could be developed that reverse stem cell dysfunctions, so that people could receive their own treated stem cells to improve diabetic wound healing.

➤ **Develop cell-based therapies.**

An intriguing prospect is the treatment of cardiovascular complications by the formation of new vessels *in vitro*. The development of a hierarchical vascular tree formed by instructing the stem/progenitor cells using extracellular materials and physical signals could overcome the poor revascularization seen in diabetes. Cell-based therapies may also come from reprogramming of cells from people with diabetes. Though iPS cells created with current protocols are unlikely to be transferred to people for treatment, these protocols offer the opportunity to take skin cells from individuals, direct their differentiation into relevant tissues, such as blood vessels, nerves, and glomeruli, and then study the sequelae of diabetes on those tissues.

Biomarkers, Imaging, and Bioinformatics

Translation of the knowledge of the molecular consequences of diabetes to effective therapies requires better measures of disease progression, faithful models of the pathology, and application of cutting-edge technologies. Validated biomarkers and surrogate end points will allow rapid screening of clinical interventions prior to larger clinical trials, and can assess risk factors and treatment adequacy for patients. Surrogate end points, if adequately validated as predictors, could enable shorter randomized clinical trials and require smaller sample sizes, factors that would accelerate acquisition of clinical information. The challenge is finding biomarkers that reliably characterize risk or the disease state among numerous biomarker candidates. Animal models exist or can be developed for specific aspects of diabetes complications, but cannot completely replicate the human clinical disease. Ready access to human samples and noninvasive imaging would allow testing hypotheses within the complexity of real people with diabetes. Without question, future advances in diabetes complications will come from emerging technologies and those not yet imagined. Currently, the field is poised to benefit from new imaging methods, systems biology approaches, and bioinformatics tools.

Key Questions

- **Can early diabetes-induced changes in tissues and organs be detected by noninvasive imaging?**
- **Will computational models that incorporate several biomarkers and imaging results create a composite analysis that is a better measure of disease progression than the individual components?**

- **What are the indicators that predict an irreversible step in the progression of diabetes complications, such as the identification of a vulnerable atherosclerotic plaque that is likely to rupture?**
- **Why do agents that prevent the onset of diabetes complications in rodent models not prevent complications progression in humans? Are intermediate models, such as swine or nonhuman primates, key steps in paths to translation?**
- **How can the large amount of data generated by genomic, epigenomic, and high-throughput screening experiments be synthesized into new, testable hypotheses on diabetes complications?**

Future Directions

➤ **Develop biomarkers for diabetes complications.**

Biomarkers are urgently needed for the early pre-clinical stages through the late end-organ failure stages of diabetes complications and for the short- (1 to 3 months) and medium-term (12 to 24 months) responses to therapies. These biomarkers should aim to be specific for the tissue and the nature of the metabolic and cellular response, and have predictive value for the risk of a given complication developing and/or progressing. Examples of biomarkers that can be pursued include:

- **Nerve fiber density in skin biopsies and visualization of corneal sensory nerves** as measures of peripheral sensory neuropathy.
- **Collagen-linked fluorescence in the skin** as an independent predictor of complications.
- **Urine exosomes and adiponectin levels** for diabetic nephropathy.

➤ **Leverage technological advances in noninvasive imaging.**

Exciting new techniques for noninvasive imaging can detect and measure changes occurring early in the development of complications before established diagnostic methods or clinical signs. A variety of imaging methods are being developed or validated for diabetes complications. Examples include:

- **Positron emission tomography (PET) imaging** to quantify regional and global myocardial blood flow and substrate metabolism, measure myocardial oxygen consumption, and detect and quantify regional sympathetic denervation of the heart.
- **Positron emission tomography (PET) with F-18 FDG** to identify inflammatory atherosclerotic plaques in the aorta and carotids.
- **Molecular imaging** using radionuclides, magnetic resonance, and optical platforms with probes targeting proteins, enzymes, or receptors involved in diabetic complications, such as RAGE and integrins (angiogenesis).
- **Tissue Doppler imaging** for diastolic dysfunction as part of cardiomyopathy.
- **Magnetic resonance imaging (MRI)** for retinal vessel damage and cardiomyopathy.
- **Voxel-based morphometry using MRI** for CNS gray and white matter measurements and peripheral nerve injury.
- **Functional MRI (fMRI)** for CNS function.
- **Dynamic contrast-enhanced MRI** for blood-retinal barrier damage and retinal oxygenation.

- ^{1}**H-magnetic resonance spectroscopy** for metabolic imaging of fatty acid content in the heart and anti-oxidant content in the CNS.
- **Near-infrared spectroscopy** for muscle oxygenation.

➤ **Improve animal and cell models.**

Considerable progress is ongoing in understanding diabetes complications through the use of rodent models. The NIH-funded Animal Models of Diabetic Complications Consortium and the Mouse Metabolic Phenotyping Centers have supported research critical in determining the fundamental causes of diabetic complications. As the molecular basis of human genetic risk factors underlying diabetic nephropathy, retinopathy, and neuropathy is elucidated, transgenic and inducible knockout mouse models can be created to evaluate the role of these genes and test drugs for newly identified pathways. A barrier to understanding diabetes-specific factors in cardiovascular disease is the lack of a good rodent model, though a well-coordinated effort in this area may lead to success. Nonetheless, rodent models have their limitations because they inadequately recapitulate the human condition and have not proven to be useful pre-clinical guides for drug development. Outbred mouse models may better represent human genetics compared to the highly inbred rodent models commonly used. Also, models that incorporate multiple aspects of the diabetic condition, rather than reductionist approaches, may produce more accurate models and could be particularly useful for understanding complications with multiple pathogenic mechanisms, such as diabetic neuropathy. Large animal models for diabetes exist and should be further developed, particularly for testing of agents in late stage pre-clinical assessments. Other models, such as *Drosophila* and zebrafish, are being developed to assess the effect of diabetes on specific aspects of complications, such as oxidant stress and angiogenesis. Improvements in cell and tissue culture techniques can also be employed for the study of diabetes complications. In particular, conditional immortalization of human cells may more closely mimic normal cell metabolism than animal models.

➤ **Transform high-throughput screening to elucidate the complexity of diabetes complications.**

Diabetes complications arise at the molecular, cell, and tissue level, so novel high-throughput assays are needed to encompass these interactions. Macromolecules that are produced or depleted by molecular pathways need to be characterized, identified, and quantified. Functional readouts and non-destructive assays are being developed for direct insight into the dynamics of cell-cell and cell-matrix interactions. High-throughput screening of cell differentiation factors can be performed on ECM microarray platforms for the culture of patterned cells atop combinatorial matrix mixtures.

➤ **Apply systems biology and bioinformatics tools to the analysis of data generated on human samples and experimental models.**

Systems biology approaches will likely show dynamic interactions and network-linked elements in different cells and conditions. Bioinformatics tools use computation, rather than intuition, to discern patterns and identify dysregulated pathways from large amounts of data. An informatics approach using pathway analyses, regulatory modules, and clustering algorithms has proven useful in the analysis of gene expression in healthy and diseased renal biopsy tissue by placing the genes in the context of regulatory elements of cellular pathways. Complexity is the hallmark of diabetes

complications. Recent advances in systems biology and bioinformatics tools may provide a new opportunity to grasp this complexity (see also the chapter, "Type 2 Diabetes As a Multi-Dimensional Disease").

Therapeutic and Preventive Strategies

Therapies are desperately needed for the prevention and the treatment of diabetes complications. A worthy goal has been and continues to be a therapy that prevents the damaging effects of hyperglycemia for multiple complications in the broad diabetes population. Another direction is stabilizing or reversing tissue-specific manifestations, such as retinal neovascularization, glomerular sclerosis, and unstable atheromatous plaques. Both of these endeavors will benefit from the study of the diversity of the individual human response to diabetes through the use of technological breakthroughs in biologic measurements. In the search for treatments for diabetes complications, success may come through the individual rather than the universal and the specific rather than the global. Individually-tailored therapy for specific complications is a goal with enormous public health and patient benefit.

Key Questions

- **Do treatments that prevent the development of complications also prevent the progression of complications?**
- **What is the impact of diabetes duration and pre-existing tissue damage on the ability to respond to therapies?**
- **What behavioral interventions improve diabetes self-management and prevent complications?**
- **Will combination therapies be more effective than single therapies? Can mechanisms for testing combination therapies be developed?**
- **What are approaches that will lead to individualizing therapies? For example, which diabetic individuals will benefit from a therapy that uncouples oxidant and carbonyl stress from hyperglycemia?**
- **How can therapies be targeted to specific tissues?**

Future Directions

➤ **Personalize drug development and treatment.**

One explanation for the discordant response of agents that treat complications in rodents versus humans is that deleterious pathways that are responsive to a certain drug may be widely expressed in inbred animal models, but expressed in only a small number of individuals. Pharmacogenomic, pharmacometabolomic, and pharmacoproteomic approaches could be used to identify markers for people who would be responsive to specific agents, such as the case for haptoglobin genotypes and responses to vitamin E therapy. In addition, genotyping of individuals participating in clinical trials through networks such as the DRCR.net can provide information on the relationship between a genetic profile and the likely response to a particular therapy. A better understanding of an individual's response to diabetes and his or her risk for complications could lead to tailoring specific therapies.

➤ **Improve behavioral approaches to treating comorbid depression and diabetes.**

Behavioral interventions can reduce depressive symptoms in people with depression and diabetes, but their effectiveness to improve metabolic control is inconsistent. Development of interventions that integrate depression treatment and diabetes self-management training for individuals with comorbid diabetes and depression is important because these people have poor adherence to their diabetes regimen and are at a greater risk for vascular complications. Research will need to address the appropriate sequence of administration of a combined depression intervention, the timing of initial and follow-up interventions, and the behavioral and biological mechanisms through which the interventions work. Effective interventions will then need to be translated into existing health plan structures and clinical care settings.

➤ **Identify novel therapeutic targets and develop more effective approaches for the prevention and treatment of diabetic complications.**

- **Cardiac steatosis.** Cardiac dysfunction in diabetes is associated with myocardial lipid accumulation. Increasing export of lipids from cardiomyocytes through activation of target proteins involved in fatty acid export may reduce the lipid accumulation and reverse the cardiac dysfunction.
- **Intracellular reactive oxygen species (ROS).** New therapies that can reduce ROS levels through decreased production or increased clearance could attack a critical early step in complications.
- **Glycation of ECM proteins.** Agents that could reverse glycation may help prevent the sclerotic process in the kidney and vascular tree and improve the ability of a tissue to respond to other therapies.
- **Anti-inflammatory agents.** Inflammation plays a key role in the development of diabetes and complications. Novel anti-inflammatory approaches that act on diabetes-related inflammation may prevent the progression of complications. A promising target for further investigation is RAGE, because blockade of this pathway in animal models shows protection from diabetes and inflammation.
- **VEGF-independent treatments for diabetic macular edema.** Anti-VEGF drugs show considerable promise for restoring vision for macular edema and proliferative retinopathy. However, successful therapy may require multiple targets that vary between individuals or over time in the same person.
- **Metabolic memory.** Therapies targeted at molecular mechanisms underlying metabolic memory could provide novel strategies to reduce the development of complications. For example, epigenetic changes might be targeted, possibly through inhibition of key histone methylases. In conjunction with inhibitors of AGEs, inflammation, and ROS, these therapies may prove effective in people who are prone to complications despite glycemic control.

➤ **Target therapies to specific compartments.**

Therapies such as VEGF that might benefit vascular regeneration in the limb could worsen retinopathy. Strategies to target drug delivery could include delayed-release preparations that can be administered locally, and the use of tissue-restricted receptors to facilitate drug uptake. Topical or transdermal therapies are needed for improving diabetic wound healing and, as for any topical application, issues of poor penetration and transient activity will need to be resolved.

- **Establish a mechanism for early evaluation of therapeutic agents parallel to the pharmaceutical industry.**

Mechanisms should be established to support research on drug and biologic development that will not be supported by industry. For example, expansion of programs such as the NIH-supported Type 1 Diabetes Rapid Access to Intervention Development (T1D-RAID) program, and establishment of clinical trial networks, would allow potential therapies to be developed and tested in early Phase I and II trials that could lead to NIH or industry supported Phase III trials to ultimately gain FDA approval.

IMPORTANCE OF RESEARCH GOALS AND STRATEGIES: HOW TRANSLATING RESEARCH OUTCOMES MAY LEAD TO IMPROVEMENTS IN HEALTH

Research focused on the reduction or elimination of diabetes complications has the potential to relieve much of the health and financial burden diabetes imposes on individuals, their families, and the Nation. A better understanding of the molecular effects of diabetes on cells and tissues will identify targets that can be used to develop new drugs that will ameliorate these harmful effects of diabetes. Genetic research is a complementary approach to finding pathways that retard or accelerate the development of complications and may lead to personalized therapies. Diabetes is a systemic disease; research on the different tissues will tailor therapies to overcome their unique properties and vulnerabilities. Research on the regeneration of cells and tissues that are damaged from diabetes has the potential for the development of a therapy that would supplement the healing process that is also impaired in diabetes. Diabetes complications are variable and have complex, often multi-factorial origins, and will benefit from research technologies that allow the interpretation of complex data on the cellular effects of diabetes and the individual responses to the disease. By harnessing research knowledge and technology in many fields, the hope is to more quickly see an array of preventive approaches and treatments that address the individual needs of the millions of people at risk for or living with diabetes complications.