Brain
Liver
Beta Cell
Kidney
Intestine
Immune Cell
Fat
Vascular Endothelium
Skeletal Muscle

From complex intercommunications that span molecules, signaling networks, cells, and tissues, and the whole human body (bottom) to the interplay of multiple organs and tissues involved in this disease (top), type 2 diabetes is a disease of many parts and many dimensions. Identifying all the changes that occur during development and progression of type 2 diabetes will be aided by systems biology approaches.

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TYPE 2 DIABETES AS A MULTI-DIMENSIONAL DISEASE

contents:

INTRODUCTION

Type 2 diabetes is not a single, "simple" disease. Rather, it is a constellation of disease syndromes, all leading to a final common diagnostic marker—hyperglycemia, or high levels of glucose in the blood. This heterogeneity and degree of complexity makes research on the causes of type 2 diabetes very challenging. Maintaining normal glucose levels (glucose homeostasis) requires a balance between insulin secretion from the pancreatic beta cells and insulin action in target tissues, which determines the rates of glucose production from the liver and glucose utilization by skeletal muscle and other tissues. Resistance to insulin action is a characteristic metabolic defect that precedes hyperglycemia in the great majority of people with type 2 diabetes and often defines a state called pre-diabetes. In people who develop hyperglycemia, the proximate cause is a decline in beta cell function, which in the face of insulin resistance leads to relative insulin deficiency.

It is important to recognize the significant interconnections among type 2 diabetes, metabolic syndrome, and cardiovascular disease. Insulin resistance is a central component in the pathogenesis of all of these conditions. More importantly, at a clinical level, both type 2 diabetes and metabolic syndrome are major predisposing factors for atherosclerosis and cardiovascular disease. Cardiovascular disease is the major cause of death in people with type 2 diabetes. Dissecting the pathogenesis of type 2 diabetes and finding new points to intervene with therapy can thus have a major impact on atherosclerosis and cardiovascular disease not just for people with diabetes, but for other at-risk individuals as well.

Multiple biological systems appear to be involved in the progressive pathogenesis of type 2 diabetes, including a variety of circulating hormones, nutrient pathways and intracellular molecular signals. These systems are controlled by a combination of genetic and environmental factors, creating numerous opportunities for disruption of function. Nearly 40 genetic loci are known to have an impact on the risk of developing type 2 diabetes. Diabetes incidence has increased dramatically in recent years, mainly as a result of environmental factors, including the current epidemic of obesity caused in part by widespread dietary changes and sedentary lifestyle. These environmental factors unmask the genetic susceptibility to diabetes that would go undetected in other environments. The complex pathogenesis of type 2 diabetes varies from person to person and reflects heterogeneous genetic and environmental triggers and metabolic abnormalities in multiple organ systems (e.g., muscle, liver, fat, beta cells, and brain). If optimal therapy is to be developed for individuals with type 2 diabetes, research approaches are needed that can systematically untangle the diverse contributors to this disease.

A systems biology approach to diabetes research recognizes this complex pathogenesis and the fact that diabetes reflects a disruption of energy homeostasis of the entire body. It takes advantage of established molecular, cellular and physiological technologies, as well as the many recently developed high-throughput discovery tools that collect and integrate large data sets obtained using "omics" technologies—genomics, transcriptomics, proteomics, lipidomics, and

metabolomics. Together, these approaches provide an opportunity to understand phenotypic variability at an unprecedented depth and to identify novel pathways of disease development and progression. With the complete sequencing of the mouse and human genomes and identification of a large number of single nucleotide polymorphisms (SNPs) that span these genomes at close intervals, disease phenotypes can now be mapped to specific regions on chromosomes. These technologies allow researchers to associate classical clinical markers of disease phenotype, such as blood glucose or insulin levels, with a much more detailed set of phenotypic variables, including tissue mRNA levels and circulating protein and metabolite concentrations. Like disease traits, transcript, protein, and metabolite levels can be used to map quantitative trait loci (QTLs) to specific regions of the genome. Ultimately, such a detailed multi-dimensional analysis could provide a roadmap for more precise tailoring of diabetes therapies to individuals who have the disease.

This chapter focuses on the great promise of a systems biology approach for understanding mechanisms leading to type 2 diabetes, for sub-classification of different forms of diabetes to assist in tailoring of therapeutic strategies, and for more detailed evaluation of new drugs to treat the disease. Integrating different areas of type 2 diabetes research is key to this approach. These areas include cell biology of hormone action, mechanisms of obesity-associated metabolic dysfunction, inflammation and endoplasmic reticulum stress, mitochondrial metabolism, gene and environmental interactions, the role of the gut microbiome, the role of the central nervous system in metabolic regulation, and opportunities for translation of new scientific discoveries from the bench to the bedside.

RECENT RESEARCH ADVANCES

High-Throughput "Omics" Technologies Yield Detailed Molecular Phenotyping: Researchers can now sequence the full expressed genome, as well as promoter regions, in large numbers of individuals and obtain measures of gene copy number and SNP maps for expression QTL assessment. Simultaneously, technologies have evolved to estimate gene function via expression of thousands of specific mRNAs at a high level of throughput and sophistication. Advances in proteomics make it feasible to simultaneously monitor several thousand tissue and plasma proteins. Disease state can influence post-translational modifications, such as protein phosphorylation, acetylation, glycosylation and other changes that can also be measured in a high-throughput manner. Metabolomics, the simultaneous quantitative measurement of thousands of small molecules, has been integrated with genotyping and transcriptomic profiling in animal and human studies. For example, in the KORA study (a population-based study from Germany), significant associations were observed between SNPs and changes in specific metabolites. This finding allowed polymorphisms in four genes encoding metabolic enzymes to be linked to perturbations in the metabolic pathways in which the enzymes function. Thus, tools are now in place to describe an individual's metabolic

state with molecular and transcriptional fingerprints and link it to the individual's genetic profile. These are important steps toward understanding the many genetic and environmental contributors to diabetes and developing personalized therapies.

Genetic Factors in Type 2 Diabetes Pathogenesis:

Although a number of rare monogenic forms of type 2 diabetes have been identified, the common forms of type 2 diabetes appear to result from complex interactions among multiple genetic loci and environmental factors. While more than 50 genetic loci associated with type 2 diabetes and obesity have recently been identified using high-throughput genome-wide association (GWA) studies, family history for diabetes still conveys more information about diabetes risk than specific gene mutations, indicating that much remains to be learned. Continued investigation of these multiple genetic factors may point to new mechanisms involved in disease risk and potential strategies for therapy (see the "Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications" chapter for additional information about advances in diabetes genetics).

Environmental Factors in Type 2 Diabetes Pathogenesis:

The most obvious environmental factors that lead to insulin resistance and the development of diabetes are a high-calorie diet, sedentary lifestyle, and obesity. It is not easy in the face of these strong risk factors to identify other, more subtle environmental risks. In addition, these factors are overlaid by cultural features that influence diet and exercise and how the individual interfaces with his or her environment. Clearly, these interactions exist and need to be identified. Conversely, regular exercise training has long been known to enhance insulin sensitivity in people with and without diabetes. In recent years, the discovery of exercise-response

genes has provided molecular insight into novel and conventional pathways that regulate glucose and energy homeostasis in health and diabetes. Exercise has acute effects to increase insulin sensitivity and enhance glucose and lipid metabolism, and training also modifies expression of genes involved in glucose homeostasis. Evidence is emerging that genetic or environmental factors may influence an individual's response to exercise and other environmental factors. Researchers have found, for example, that antioxidants may block the beneficial effect of exercise training on glucose metabolism. Proper integration of these factors is needed to define exercise training programs that promote the greatest health benefits, including enhanced insulin sensitivity in diabetic humans.

Intracellular and Extracellular Signaling Pathways That Integrate Growth Factor, Nutrient, and Energy Sensors:

Elucidation of the role of receptors, G proteins, pathways of insulin and other hormone signaling, protein kinases and phosphatases, and histone acetylases and deacetylases has allowed for a more thorough understanding of signal transduction pathways that govern metabolism. Important advances include elucidation of pathways involving insulin receptor signaling, phosphatidylinositol (PI) 3-kinase, the serine/threonine kinase Akt, mitogen-activated protein (MAP) kinases, forkhead transcription factors, and sirtuins, as well as the mechanisms by which they are integrated, including transcriptional co-activators and co-repressors. Modern mouse genetic engineering has allowed the function of each of these molecules to be defined *in vivo*, as well as *in vitro*. In addition to hormones, nutrients have been shown to directly modify signal transduction pathways that regulate glucose and lipid metabolism through kinase cascades (the modification of proteins, lipids and other molecules by the addition or removal of phosphate groups). Recent

advances include the identification and characterization of protein kinase cascades, such as the AMP-activated protein kinase (AMPK) and the amino acid sensor mammalian target of rapamycin (mTOR), both of which are also regulated by hormones. These discoveries emphasize the important role of nutrients on cellular and whole body insulin action through an interplay between intercellular energy sensors and metabolic/gene regulatory events.

Tissue Crosstalk and Feedback in Controlling Energy Balance:
Over the past decade, a whole new class of hormones has been discovered—the "tissuekines." These circulating bioactive peptides are secreted from leukocytes (cytokines), different fat depots (adipokines), and muscle (myokines), for example, and act on liver, brain, and other tissues. The activities of these tissuekines revealed a previously unsuspected level of crosstalk between cell types throughout the body that contributes to metabolic regulation. Crosstalk between tissues is also mediated by nutrients and metabolites, particularly circulating lipid and carbohydrate species generated from liver and fat.

Transcriptional Mechanisms Regulating Hepatic Glucose and Lipid Metabolism:
In addition to regulation of membrane receptors and transporters, hormones and nutrients can regulate cellular function through control of gene expression. Advances in the molecular biology of nuclear receptors and other transcription factors have furthered understanding of protein complexes involved in the regulation of gene expression in fat, liver, and muscle, as well as other tissues, that affect overall metabolic status and cellular functions in highly-differentiated cell types. Nuclear receptors involved in diabetes pathogenesis can be direct targets of steroid and thyroid hormones, as well as targets of nutrient signals and of xenobiotics—

chemical substances not normally found in the organism but introduced from the environment. A major part of insulin action also occurs by regulation of gene transcription by forkhead box O1 (FoxO1) and other transcription factors. These effects are further modified by co-repressors and co-activators, as well as covalent modification by acetylation/deacetylation, in response to external stimuli. Over the past decade, research on the contribution of transcriptional control to overall metabolic fuel homeostasis has provided important insights into fundamental mechanisms of hormone action, control of cell differentiation, and the biological clocks that govern many bodily functions.

Cell Biology of Protein Trafficking and When It Goes Wrong:
Insights into the mechanisms that control protein trafficking among organelles in cells yield new information about hormonal regulation of glucose metabolism, particularly control of insulin secretion within the beta cell and control of glucose transport in muscle and fat cells. Scientists have identified many of the proteins and their interactions by which insulin regulates GLUT4 glucose transporter trafficking from intracellular regions to the cell membrane to stimulate glucose uptake. Defects in insulin-stimulated glucose uptake in muscle represent one of the earliest detectable lesions in humans with pre-diabetes.

The endoplasmic reticulum (ER) is a vast network of membranes in which all secretory and membrane proteins are assembled, and proper folding, maturation, storage, and transport of these proteins take place. ER stress has been detected in both experimental and human obesity and in states of insulin resistance, such as fatty liver associated with type 2 diabetes. In this condition, misfolded proteins or metabolic signals activate a complex series of events called the unfolded protein response (UPR)—an attempt to restore the

functional integrity of the ER. Inflammatory pathways involving the enzymes c-Jun N-terminal kinase (JNK) and IkappaB kinase (IKK), reactive oxygen species, and other nutrient sensing and pathogen response systems are also integrated with ER function. Molecular and chemical tools that can mitigate ER dysfunction have demonstrated some therapeutic efficacy in rodent models of obesity, insulin resistance, and type 2 diabetes. ER stress is implicated in beta cell dysfunction and viability, suggesting that it could also be an important feature of type 1 diabetes.

Inflammation and Metabolic Homeostasis:

Experimental, epidemiological, and clinical evidence produced in the past decade has causally linked inflammation and the inflammatory response to the pathogenesis of obesity, type 2 diabetes, and metabolic syndrome, and their complications. Several important molecular mediators, including the stress kinases JNK and IKK, and the suppressors of cytokine signaling (SOCS proteins), have been identified and genetically validated in mouse models. Interventions that suppress or modify the inflammatory response systems have been shown to improve insulin sensitivity and glucose metabolism, demonstrating that inflammation as it occurs in diabetes participates in metabolic deterioration. Most recently, clinical studies have shown similar effects, providing evidence for a role of inflammation in human metabolic disease. It has also become clear that inflammation plays an important role in diabetes complications, particularly macrovascular disease, where the process can involve both vascular endothelial cells and smooth muscle cells.

Mitochondrial Activity and Insulin Resistance in Humans and Animal Models:

Multiple studies in humans using noninvasive imaging and muscle biopsies indicate that impaired capacity for oxidative function is present in type 2 diabetes and insulin resistance. Researchers observed that the insulin resistance present in people who have type 2 diabetes or are undergoing normal aging is associated with reduced activity of key mitochondrial pathways that use oxygen to generate adenosine 5'-triphosphate (ATP), the cell's "energy currency"—leading to the idea that muscle mitochondrial oxidative dysfunction may be an early defect in diabetes. Coordinated changes in expression of nuclear genes encoding mitochondrial proteins underlie these changes. Researchers can now explore a variety of mechanisms potentially linking impaired mitochondrial oxidative function to insulin resistance, including excessive lipid accumulation over time, particularly with ensuing energy excess, leading to lipotoxicity; reduced ATP synthesis for energy-requiring insulin signaling and insulin-stimulated glucose uptake; reduced ATP synthesis during exercise, potentially contributing to reduced aerobic capacity, muscle fatigue, and decreased voluntary exercise over time; and, in adipose cells, reduced insulin signaling and reduced secretion of adipokines.

Circulating Fatty Acids, Ectopic Lipid Storage, and Insulin Resistance:

Chronic exposure of the liver to elevated fatty acids overwhelms the capacity for fatty acid oxidation, leading to esterification in triglycerides, increased production of very low density lipoproteins (VLDL), and generation of lipid-derived metabolites that interfere with insulin signaling. Similar events occur in muscle, where chronically elevated lipids increase fatty acid beta-oxidation enzymes. In sedentary humans and animals, the induction of the beta-oxidative machinery ultimately leads to the activation of enzymes that interfere with insulin action. It appears that elevated circulating lipids and inappropriate fat storage in peripheral tissues (ectopic fat) occur when the adipocyte cannot store all of the fat being produced.

The discovery that lipid droplet biogenesis proteins modulate insulin sensitivity reinforces the hypothesis that mechanisms that impair fat storage in adipocytes lead to insulin resistance. This hypothesis is further supported by findings of insulin resistance induced by infusion of exogenous lipid, as well as insulin resistance in lipodystrophy, a condition characterized by abnormal fat distribution.

Amino Acids Play a Role in Insulin Resistance and Glucose Homeostasis:

Obese and insulin resistant humans have elevated levels of various amino acids, including the branched-chain amino acids (BCAA). One BCAA, leucine, interferes with insulin action via stimulation of the enzymes mTOR and S6K1 and modification of insulin receptor substrate (IRS) proteins. A comprehensive targeted metabolomics study in obese, insulin-resistant individuals and lean controls revealed that a BCAA metabolite signature was strongly related to insulin resistance in this cohort. These findings, coupled with rodent and human feeding and amino acid infusion studies, show that during high fat consumption, BCAA could contribute independently to development of obesity-associated insulin resistance. A study in mice, which correlated genetic loci, transcripts, and metabolites, identified a molecular network through which the amino acid glutamine regulates phosphoenolpyruvate carboxykinase expression and, therefore, glucose production in the liver. These studies illustrate how nutrients and metabolites can have broad effects on multiple metabolic pathways and point to the need for more careful dissection of the role of individual nutrients and metabolites in the pathogenesis and possible treatment of type 2 diabetes and other insulin resistant states.

Novel Locations and Functions of "Taste" Receptors and Their Potential Impact on Diabetes and Obesity:

Novel nutrient receptors are being identified throughout the body. For example, the sweet and bitter taste receptors of the oral cavity that underlie taste quality perception are also distributed throughout the specialized endocrine cells of the gastrointestinal tract and pancreas. Recent studies have shown that the gut-expressed sweet taste receptor (and its associated signal transduction pathway) helps regulate intestinal uptake and metabolism of sweet compounds, and the release of insulin from the pancreas during digestion. In other studies, the oral taste cells have been shown to contain hormones previously found in gut endocrine cells that are known to regulate insulin release from the pancreas. These non-traditional functions of the oral and gut sweet receptors suggest a direct role for taste and taste receptors in diabetes and obesity, a role that is markedly different from the sensory function of the oral taste system. Research has also revealed an association between bitter taste receptors in the gut and glucose metabolism. Further study of the roles of nutrient receptors in diabetes and obesity is clearly warranted.

Role of Brain in Carbohydrate Metabolism:

The last 10 years have provided tremendous advances in understanding central nervous system (CNS)-dependent control of metabolism. Initial studies in mice and rats with inactivation of the insulin signaling cascade in the brain revealed a critical role for neuronal insulin action in control of hepatic glucose production. Subsequent studies uncovered control of both hepatic glucose production and peripheral glucose disposal, as well as adipose tissue mass, by hormones acting via the CNS,

including leptin, insulin, ghrelin, glucagon-like peptide-1 (GLP-1), and others. The growing understanding of the primary neuronal target sites, their projection sites, and the intracellular signaling cascades mediating these effects provides promising novel avenues for the treatment of impaired glucose metabolism and associated disorders, such as dyslipidemia.

Bench to Bedside and Back Again: Research on the mechanism of action of drugs for type 2 diabetes treatment has yielded tremendous insight into basic biology, just as knowledge gained through basic studies has led to powerful new classes of drugs. Metformin, a biguanide insulin sensitizer, suppresses hepatic glucose production and lowers fasting glucose. This effect appears to be, at least partly, through the action of AMP-activated protein kinase, an important energy sensing protein kinase that regulates nutrient metabolism. The second class of insulin-sensitizing diabetes drugs, the thiazolidinediones, act by binding to the peroxisome proliferator-activated receptors (PPAR), primarily in the nucleus of fat cells, regulating insulin sensitive genes for nutrient metabolism and fat storage. Exenatide, or exendin-4, is a new peptide therapeutic that was approved to treat type 2 diabetes in 2005. This drug, which was obtained originally from the venom of the Gila monster, acts to stimulate secretion of insulin from the beta cell by mimicking the action of

the normal incretin GLP-1. Exendin-4 is more effective than GLP-1 itself because it resists degradation and has a much longer half-life in the circulation. However, now drugs are also available that inhibit the enzyme that normally degrades GLP-1, an enzyme called dipeptidyl peptidase-4 (DPP-4), and these serve as an oral alternative to the peptides, which require injection. These examples demonstrate the synergy between basic mechanistic research, discovery research, and drug development. They support the hope that additional drugs will be developed to target molecules involved in diabetes pathophysiology, and that biological pathways will be elucidated through the investigation of new bioactive molecules. The huge promise of a systems biology approach to diabetes research is that it has the potential to identify factors and pathways that can be targeted for a more holistic diabetes therapy.

Gastric Bypass and Glycemic Control: While initially aimed at the treatment of obesity, gastric bypass and other bariatric surgeries have been shown to produce a much greater improvement of glucose metabolism than expected from the obtained weight reduction. Diabetes is resolved in some cases after surgery but prior to significant weight loss. Identifying the molecular basis of the gut/CNS crosstalk responsible for these effects is expected to set the ground for the development of novel therapeutic strategies for diabetes.

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 cell signaling and cell regulation as an important area of opportunity in understanding type 2 diabetes, and acknowledged the emergence of new technologies that were already propelling the field. Since then and with the support of the NIH, many of these new technologies have come to fruition so that now a true systems biology approach to type 2 diabetes is feasible. This will allow elucidation of the complex biological interactions involved in disease pathogenesis by integrating information about cell signaling pathways with genetic, transcriptional, metabolic, and environmental factors. Described below are research questions and opportunities focused on developing an integrated understanding of the molecular pathogenesis and consequences of type 2 diabetes.

Insights into Gene and Environment Interactions in Type 2 Diabetes

The rise in diabetes incidence over the recent decades underscores the existence of environmental triggers that promote disease in people with a genetic predisposition. Researchers understand some of these triggers: the "unhealthy diet" and sedentary lifestyle that result in obesity are major risk factors for type 2 diabetes, and maternal obesity and gestational diabetes can result in metabolic abnormalities in the offspring. Unfortunately, in contrast to "omic" technologies that allow a relatively complete sampling of genetic, genomic, proteomic, and metabolomic variables, at present there is no similar unbiased approach available for identifying all of the environmental stressors that might contribute to

diabetes. However, recent studies have shown that epigenetic modifications of DNA structure can take place as a result of environmental factors that act in addition to the mutations found in inherited DNA sequences. Researchers have also begun to investigate how nutrients and exercise alter gene transcriptional programs, and explore novel transcriptional and translational regulatory mechanisms, such as noncoding microRNAs.

Key Questions

- **What can a systems biology approach reveal about gene and environment interactions?**

- **Can a systems biology approach reveal the mechanisms whereby the identified diabetes genes and gene variants contribute to glucose homeostasis and type 2 diabetes pathogenesis?**

- **Do some genes and genetic variants contribute to diabetes only when a particular environmental factor is present?**

- **What is the mechanism by which diet, exercise, and other environmental factors affect insulin sensitivity, and what are the specific dietary and other environmental factors that are most important?**

- **How does the intrauterine and early postnatal environment influence diabetes risk?**

- **How do epigenetic modifications in gene expression profiles influence the metabolic response in type 2 diabetes?**

- **What other major regulatory mechanisms, such as miRNA, influence diabetes pathogenesis?**

Future Directions

➤ **Develop new tools and technologies for complex genetic/genomic studies and metabolic profiling.**

Once established, core laboratories focused on the various "omic" technologies can take on the challenge of determining the role of changes at these levels in various metabolic and diabetic states. This service requires policies that ensure sufficient time and resources for continued technology development, which is critical for future new systems biology approaches. Advanced functional genomic platforms can help researchers characterize model organisms and animal models in search of specific gene-environment interactions that lead to loss of metabolic homeostasis and diabetes. Development of new tools and technologies for large-scale population genetics and the emerging interface between epidemiology and high-density genetic and molecular analysis will lead to better understanding of how genetic variation determines changes in gene networks and metabolic function in clinical populations. High-content molecular profiling technologies (metabolomics and proteomics) coupled with sophisticated computational and bioinformatics tools are needed to define novel pathways that can be exploited for drug discovery and diabetes therapy. Further development of computational tools for integration and interpretation of large, complex data sets emanating from different "omics" platforms should also be undertaken. Aligning these activities with educational and training opportunities in mass spectrometry, NMR, data analysis, and advanced computational methods will help scientists realize maximal benefit and impact from application of these technologies to diabetes research.

➤ **Study the role of microRNAs in type 2 diabetes pathogenesis.**

Research is needed to discover and quantitate the universally present small non-coding RNAs or microRNAs (miRNAs) present in all tissues and cell types. Several studies have begun to provide evidence that small RNA species may play a role in the pathogenesis of type 2 diabetes and that either endogenous miRNA or their specific inhibitors may be exploited as targets for therapeutic intervention. The full impact of miRNAs and how they modify physiology and interact with other genetic factors remain to be defined.

➤ **Investigate the role of epigenetic changes in type 2 diabetes pathogenesis.**

Epigenetic changes in DNA have been noted in certain forms of cancer and during embryologic development. Accumulating evidence suggests that DNA methylation can be modulated by the environment. For example, glucocorticoid receptor gene methylation is modified in the hippocampus of rat pups in response to maternal grooming. This observation provides a direct example of how childhood environment may determine adult stress-responses. Dietary modification can also have a profound effect on DNA methylation and genomic imprinting. Graded methylation-driven silencing of an element that regulates coat color and the obesity phenotype of the agouti mouse has been shown in the offspring of mice fed diets with different amounts of folic acid. Environmental toxins, such as heavy metals, can disrupt DNA methylation. Estrogenic and anti-androgenic toxins that decrease male fertility can also alter DNA methylation, and these changes are inherited by subsequent generations. Thus, epigenetics may

provide a mechanism to link the changing environment to long-term effects on incidence of type 2 diabetes in individuals and families. Moreover, DNA methylation, histone acetylation, and deacetylation may introduce epigenetic changes in the intrauterine environment and throughout an individual's lifetime, which may influence age-related modifications in gene expression profiles that can contribute to metabolic disease. Identifying the enzymes that mediate modifications in DNA structure and transcription provides an opportunity for a more systematic approach to defining the role of epigenetic enzymes in control of metabolic regulation and diabetes pathogenesis.

➤ **Investigate how nutrients interact with different genetic backgrounds to influence diabetes development.**

In rodents, genetic background dramatically affects response to environmental stresses, such as high-fat diet, as well as to other genetic alterations that lead to insulin resistance or changes in beta cell function. A systems approach with measurement of transcript, protein, and metabolite levels allows the integration of both genetic background and environmental influences and the combined analysis of various "omics" information to discover regulatory pathways that may be relevant to disease progression. The identification of these pathways and distinct nutrient and energy "sensors" that control them may lead to the development of new prevention strategies for certain people in the pre-diabetic state through individualized diets that can enhance insulin action.

➤ **Identify xenobiotics and xenobiotic receptors that might act as environmental sensors and influence diabetes development.**

In addition to major dietary components, such as carbohydrates, fats, and protein, minor dietary components (trace metals) and xenobiotics, i.e., other chemicals that enter the body, such as drugs and environmental pollutants (e.g., dioxins, polychlorinated biphenyls), may have important effects on pathogenesis of diabetes and its complications. While some candidate xenobiotics and xenobiotic receptors involved in metabolism have been identified, research is needed to discover more substances that can play a role and to understand their mechanisms of action at a cellular and molecular level.

Metabolic and Hormonal Regulation in Diabetes

The loss of glucose homeostasis that defines diabetes is the clinical manifestation of the failure of an enormous system of homeostatic mechanisms involving many cell types and the huge number of intersecting signaling and metabolic pathways within each cell. Great strides have been made in understanding the cellular basis of this metabolic regulation. In addition to information gleaned from targeted molecular and cellular biology approaches, genomic, proteomic, and metabolomic approaches have revealed a number of new pathways, regulatory events, and modes of interactions among tissues, receptors, and processes. Physiological and cellular models of hormone action have revealed new molecules involved in receptor biology, protein trafficking, control of enzyme activities, and transcription. This progress has resulted in a new, integrated view and opened the door to exciting new therapeutic opportunities for diabetes.

Key Questions

- **Are there unidentified hormones, or small molecules with hormone-like activity, that signal from tissue to tissue or communicate via the CNS to affect metabolism, and what are their functions? What are their sites of production and mechanisms of action?**

- **What is the full complement of molecular players involved in regulation of gene transcription and protein translation? How do they interact to effect different gene expression patterns?**

- **How do the many signaling pathways within cells interact to control metabolism, cell growth, and energy production?**

- **What are the dominant molecular mechanisms of insulin resistance in different tissues, and how do they differentially affect glucose, lipid, and amino acid metabolism?**

- **Is the mechanism of insulin resistance different in different pathological states, such as diabetes, obesity, polycystic ovarian disease, and related conditions?**

- **What are the earliest defects that can be detected in the pathogenesis of type 2 diabetes, and are they the same in most individuals? Which of these defects are genetically programmed and which are acquired?**

Future Directions

➤ **Identify transcriptional targets of key protein kinases involved in metabolic regulation.**

Evidence indicates that numerous protein kinases, including S6K, Akt (and other serum and glucocorticoid-inducible kinase (SGK) family members), IKKs, mTOR, glycogen synthase kinase-3 (GSK-3), and MAP kinases, are key integrators of signaling to transcription factors. A variety of post-translational modifications to proteins, including phosphorylation, acetylation, and ubiquitination, directly regulate transcriptional activity, cellular location, and protein interactions. Efforts are needed to more fully understand the transcriptional mechanisms of pathway integration and identify key nodal points for effective intervention. Development of additional probes for protein components of these systems is essential for use in cellular and *in vivo* imaging.

➤ **Develop the ideal "molecular signature" of nuclear receptors and transcription factors.**

Combinatorial diversity in the components of transcriptional and other signaling complexes leads to subtle differences in gene expression patterns, particularly concerning nuclear receptors, which are established drug targets. Research to solve the structures of these complexes using x-ray crystallography and cryo-electron microscopy will help scientists identify the components of these signaling complexes and the most beneficial molecular conformations of co-activator/co-repressor complexes that generate a positive impact on *in vivo* energy balance and metabolism.

➤ **Ascertain pathways of lipid and lipoprotein synthesis and breakdown in the liver.**

Research is needed to define molecular pathways that activate the transcription factor sterol regulatory element binding protein (SREBP), and regulate cholesterol, fatty acid, and triglyceride metabolism. In particular, the role of the SREBP pathway in the pathophysiology of hepatic steatosis (fatty liver) remains poorly understood. Research to understand the relationship between ER stress pathway activation and hepatic lipogenesis—the conversion of glucose to fatty acids in the liver—is of key importance. Such studies may be able to leverage efforts in other fields, such as research on the impact of alcohol or toxins on these factors and pathways in the liver.

➤ **Elucidate molecular events involved in insulin action and insulin resistance.**

Insulin resistance is central to type 2 diabetes and the metabolic syndrome. Despite great progress in understanding insulin signaling, much remains to be learned about the normal function of this network in regulation of multiple pathways of cellular metabolism. In addition, defining the specific tissues and organs involved in different states of insulin resistance, such as obesity, type 2 diabetes, polycystic ovary disease, and others, offers the possibility of targeting specific pathways in different disorders.

➤ **Elucidate molecular events involved in insulin-stimulated glucose transport.**

Progress has been made in identifying early signaling pathways from the insulin receptor, but how these pathways regulate the trafficking of the main insulin-sensitive glucose transporter protein GLUT4 remains uncertain. Efforts are required to identify the G protein target(s) of AS160 (a substrate of the Akt serine/threonine kinase that regulates insulin-stimulated GLUT4 trafficking), the role of other kinase substrates, and the role of the exocyst and soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor, or SNARE, protein complexes. Also needed are cellular models of hormone action in muscle, especially human muscle, where some of the earliest defects in type 2 diabetes have been identified.

➤ **Elucidate mechanisms underlying regulation of circadian rhythms vis a vis metabolic control.**

Night-shift working and other forms of circadian misalignment lead to major metabolic abnormalities, including leptin and insulin resistance. These factors play an important role in some populations at high risk for diabetes. These control mechanisms may also play an important role in control of normal metabolism. Therefore, efforts are required to understand the mechanisms underlying integration of clock genes with metabolic processes.

➤ **Identify novel "tissuekines" and elucidate their role in regulation of energy balance and interplay with known hormones.**

Newly identified muscle-, bone-, and adipose-derived factors have a profound impact on metabolic pathways in other tissues. Efforts are needed to fully catalog these "tissuekines," identify their receptors and signaling pathways, and begin to understand their physiological roles. Also, more information is needed on insulin action and cross-talk between non-traditional target tissues of insulin (brain, kidney, vasculature, gastrointestinal tract, and immune cells) and traditional target tissues (muscle, fat, and liver).

Inflammation and Endoplasmic Reticulum Stress—Impact on Insulin Signaling and Glucose Metabolism

Consensus is emerging regarding some of the mechanisms resulting in insulin resistance, beta cell failure, metabolic dysregulation, and diabetic complications. Two important components are inflammation and ER stress. Inflammation in obesity, type 2 diabetes, and atherosclerosis is not the adaptive immunity that occurs in response to a broad range of extrinsic injuries. Rather, it is activation of the innate

immune system, which is characterized by abnormal cytokine production, increased acute phase reactants, and activation of a network of inflammatory and stress signaling pathways. A perhaps related phenomenon is the disruption of the integrity of cellular organelles, particularly ER. Under stress conditions triggered by misfolded proteins or metabolic signals, the ER activates the UPR, resulting in activation of the enzymes JNK and IKK and generation of reactive oxygen species, which, in turn, can modify metabolism and damage tissues.

Key Questions

- **What biological networks and molecular mechanisms initiate and govern "chronic" inflammation? Which immune cell subtypes are critical in metabolic regulation? Can the cell subtypes be manipulated to alter metabolic outcomes?**

- **What mechanisms activate inflammatory networks within non-immune cells, such as adipocytes, hepatocytes, myocytes, pancreatic beta cells, and neurons?**

- **What are the metabolic triggers and mechanisms for ER dysfunction in obesity, diabetes, fatty liver, and cardiovascular disease? Is there a link between ER and mitochondrial dysfunction? Are there biomarkers that faithfully reflect organelle failure, ER stress, or tissue inflammation?**

- **Could the inflammatory response and ER failure link metabolic homeostasis to other clustering diseases, such as asthma, neurodegeneration, cancer, and others, which are also frequently associated with obesity and/or insulin resistance?**

- **Are there chemicals that modify the function of ER or other organelles, or influence chronic inflammation? Which molecules that demonstrate pre-clinical efficacy could be tested for proof-of-principle in humans most expeditiously and effectively?**

Future Directions

➤ **Elucidate the triggers and responding cell types that initiate and maintain chronic inflammation in diabetes.**

The mechanisms leading to establishment of chronic low-grade inflammation in diabetes, including the involvement of immune and other cell types, the relationship between acute inflammatory responses and the establishment of chronic inflammation, and the identification of molecular signatures defining chronic inflammation, are important areas for research.

➤ **Investigate molecular mechanisms linking metabolism and chronic inflammation.**

Inflammation in diabetes occurs in the context of significant metabolic dysregulation. Studies are needed to define how signals emanating from metabolic pathways may exacerbate or modulate chronic inflammation, and how inflammation may exert an adverse impact on metabolism to accelerate disease progression. The role of inflammation in vascular endothelial cells and smooth muscles cells should also be investigated, as this forms an important link between diabetes, obesity, atherosclerosis, and cardiovascular disease.

➤ **Identify and develop novel targets for intervention.**

Emerging technologies are raising the possibility of identifying a wide range of new potential therapeutic targets. A thorough examination of the relationship between epigenetic change and chronic inflammation in both immune cells and the cellular targets of inflammatory damage is now feasible. Similarly, advanced chemical biology platforms and screening techniques are identifying novel small molecules that may have therapeutic promise. In all cases, the development, validation, and use of appropriate animal models, as well as early clinical research, remains key to accelerating bench to bedside translation.

➤ **Characterize the molecular mechanisms leading to ER dysfunction in diabetes.**

Efforts are needed to understand the different branches of the UPR and how these pathways interact to regulate target organ metabolism. The development of reagents to monitor ER function in cells and tissues from animals and humans, as well as reagents/methods to experimentally generate physiologically relevant forms of ER stress in cultured cell systems, would accelerate research in this area. Research is needed to characterize the ER, its resident proteins, lipids, structural and morphological properties, and folding environment, as well as the links between different inflammatory responses, mitochondrial function, and ER stress. This line of research should incorporate chemical biology platforms for small molecule discovery, genome-wide functional screens, and development of new experimental models to monitor cellular responses and live animals during the course of ER stress/metabolic disease. Finally, clinical proof-of-principle studies of promising pharmacological interventions are needed.

Mitochondrial Metabolism

Mitochondrial dysfunction has emerged as a potential explanation for the tight relationship of obesity and overnutrition with the development of insulin resistance. One hypothesis postulates that impaired fatty acid storage by a limited adipose tissue expansion capacity leads to excess circulating fatty acids. These cause insulin resistance, either as the consequence of incomplete oxidation leading to reactive oxygen species (ROS) production, or through bioactive lipid derivatives, like diacyglycerols or ceramides. Fatty acids can exert deleterious effects directly in muscle and/or indirectly through macrophages by eliciting chronic low-grade inflammation. Because the primary disposal of free fatty acids in muscle and oxidative tissues is through mitochondrial beta-oxidation, mitochondria may play a crucial role in determining individual susceptibilities to insulin resistance and diabetes. Several lines of experimentation, including noninvasive NMR and gene profiling, have discovered deficits in mitochondrial function or in the expression of mitochondrial genes in human muscle from individuals with diabetes or a family history of diabetes. Moreover, the capacity of adipose tissue to sequester free fatty acids is correlated with its mitochondrial levels and oxidative capacity. Thus, the study of mitochondrial function and its role in type 2 diabetes is an important new research avenue.

Key Questions

- **Are the changes in mitochondrial function cause or consequence of insulin resistance or an independent contributor to metabolic derangement?**
- **What mechanisms control mitochondrial levels and functional outputs in human tissues and how do these relate to the genetics of the disease?**
- **What is the relationship between mitochondrial energy metabolism and insulin signaling?**

- **Does mitochondrial dysfunction link aging to type 2 diabetes?**
- **What are the most promising approaches to treat mitochondrial dysfunction in metabolic disease?**

Future Directions

➤ **Develop animal models of mitochondrial deficiencies that more precisely mimic human physiology, and ascertain their effect on the development of insulin resistance and diabetes.**

Current animal models that are designed to evaluate the impact of mitochondrial oxidative capacity on the development of insulin resistance are largely limited to mouse whole body or muscle-specific knockdowns of co-activators or co-repressors of mitochondrial biogenesis. The pathology associated with these manipulations tends to be extreme. More subtle, inducible genetic alterations in fatty acid beta oxidation, ATP production, or ROS generation that affect specific cells, tissues, or organs may produce models that more faithfully mimic human physiology. These and similar models would be important tools for testing the hypothesis that specific defects in mitochondrial function underlie the development or progression of insulin resistance and type 2 diabetes.

➤ **Systematically assess the interactions between mitochondrial energy metabolism and insulin signaling in model systems.**

Tractable genetic systems such as *Caenorhabditis elegans* (a species of roundworm) and *Drosophila melanogaster* (a species of fruit fly) have been successfully used to delineate the basic elements of insulin signaling. However, a systematic approach to studying the interactions between mitochondrial function and insulin signaling in mammals has not been taken. The development of metrics and tools to assess mitochondrial fatty acid oxidation, ATP production, ROS production, and other mitochondrial-specific outputs in these model systems, as well as their effects on specific elements of the insulin signaling pathway, is necessary.

➤ **Discover the broader consequences of cellular mitochondrial energy metabolism on tissue function.**

Adipose and muscle tissue functions are dependent not only on the activities of the differentiated cell composing the tissue (i.e., adipocyte, myocyte), but on the factors that nourish and maintain communication between the tissue and the whole body, such as its vascular network. The interactivity between mitochondrial energy metabolism and the generation of signals necessary for the proper development of muscle and adipose tissue microvasculature is likely to be a key factor in energy homeostasis and needs to be studied.

Nutrient Role in Glucose Homeostasis—Mechanisms of Overnutrition-Driven Tissue Dysfunction

The wide availability of energy-rich fast foods in U.S. society and changes in dietary habits over the past several decades have lead to chronic overconsumption of all the major macronutrients—carbohydrate, fat, and protein—and to obesity. Obese humans and animals are often insulin resistant and exhibit increases in circulating lipids and amino acids even before the appearance of elevated glucose levels and type 2 diabetes, suggesting that imbalance in these nutrients may contribute to the ultimate development of hyperglycemia. Lipid abnormalities of obesity include

elevated levels of multiple fatty acids and triglyceride in the plasma, increased LDL ("bad") cholesterol and reduced HDL ("good") cholesterol, and accumulation of lipids in liver and muscle (ectopic fat). High fat diets, acute (6 to 12 hour) infusion of lipids, or growth of isolated muscle cells in the laboratory in the presence of high levels of fatty acids are all sufficient to cause insulin resistance. Chronic culture of islet beta cells with high levels of fatty acids or glucose impairs glucose-stimulated insulin secretion and eventually leads to activation of cell death pathways, a demonstration of the harmful effects of high concentrations of certain nutrients. On the other hand, nutrient-derived signals are important in relaying the body's energy status and partitioning nutrients between storage and use as fuel, in inter-organ communication, and in regulating glucose homeostasis. In order to break the link between obesity and diabetes, it is important to understand why insulin resistance, tissue dysfunction, and eventually diabetes can be the body's response to "too much of a good thing."

Key Questions

- **What are the key intracellular and extracellular nutrient-derived and nutrient-independent signals that trigger insulin resistance, and do humans use the same signaling pathways as animal models? In addition to macronutrient composition, which individual micronutrients play important roles?**
- **How do these nutrient-derived signals interact with genetic variability to protect or predispose people to diabetes?**
- **What are the mechanisms by which overnutrition and the overabundance of nutrient concentrations lead to impaired beta cell mass and function?**

- **What are the similarities and differences among nutrition-derived signals that mediate dysfunction in key organs and tissues involved in fuel homeostasis, including muscle, liver, vasculature, and adipose tissue?**

Future Directions

➤ **Understand the factors that regulate fat storage in the adipocyte and in non-adipose tissues.**

Fat storage in adipocytes appears to protect other cell types in the body from excess lipid storage, where it is accompanied by insulin resistance, inflammation, and mitochondrial dysfunction. Research is needed to understand how adipose fat storage is governed, and how this process fails in diabetes and obesity. Further, research on how fat accumulation in beta cells, liver, muscle, and potentially endothelial cells contributes to insulin resistance and organ dysfunction could clarify the importance of ectopic fat in diabetes pathogenesis. Such studies could reveal whether this lipotoxicity plays a role, along with hyperglycemia and hypertension, in the classic diabetic complications, such as nephropathy, retinopathy, cardiopathy, and neuropathy.

➤ **Understand the normal signaling roles of circulating lipid and other nutrient-derived molecules.**

Lipids constitute a very large family of molecules—the "lipidome"—that vary by carbon chain length, number, and positions of double bonds and attached headgroups. Many lipids appear to play signaling and regulatory roles within and between tissues. Some amino acids act as nutrient signals that regulate protein synthesis and insulin sensitivity. It is becoming clear that nutrients

and their metabolites interact with some nuclear proteins to regulate gene transcription. Therefore, the exchange of nutrients among organs and tissues seems to constitute tissue crosstalk that carries important regulatory information, and these molecules appear to be playing a much larger endocrine and paracrine role than has been previously appreciated. More knowledge of these roles may answer such questions as how the beta cell detects insulin resistance in the liver and muscle. Systems biology approaches will be useful to elucidate the complex nature of nutrient-derived signaling in fuel homeostasis and diabetes pathogenesis.

➤ **Understand the mechanisms whereby excess nutrient load leads to organ dysfunction.**

Just as chronic fat deposition in the liver and beta cell appears to interfere with tissue function, chronically elevated glucose leads to diabetic complications in most tissues, including kidney, eye, bladder, nerves, and the cardiovascular system. Acutely elevated blood nutrient concentrations also appear to have harmful effects, including insulin resistance and other functional abnormalities. Both chronic and acute effects depend on the concentrations of specific signals, on the mass effect of too much energy-dense fuel, and on the chemical properties of these nutrient molecules. These observations indicate that overnutrition can affect organ function and disease state by a variety of mechanisms, and it remains to be determined exactly what those mechanisms are, and which are most important for human health.

➤ **Develop noninvasive or minimally invasive approaches to measure nutrients and other metabolites in specific locations.**

Nutrients are found in different concentrations in different places in the body and likely have different impacts, but measurements are often confined to peripheral venous blood. The liver and brain both respond to the concentrations of insulin, glucose, and fatty acids, which vary considerably between the portal vein and brain blood vessels. Novel detection methods need to be developed, such as imaging contrast agents that are sensitive to nutrient concentration, or indwelling, miniaturized, telemetric sensors that can be used in research animals.

New Players in Control of Metabolism: Role of the Brain and the Gastrointestinal Tract

The elucidation of new roles for brain, gut, fat, and other tissues in regulating glucose metabolism constitutes one of the most exciting areas of type 2 diabetes and obesity research. This line of research has led to widespread enthusiasm for the idea that energy homeostasis is a whole-body phenomenon, and for the systems biology approach to research. In addition to governing eating behavior, the brain responds to hormone, molecular, and nutrient signals to coordinate liver glucose output, energy production and storage, and insulin secretion. The exact neuronal networks and molecular mechanisms remain to be fully described. The startling observation that type 2 diabetes can often be almost immediately resolved, even prior to significant weight loss, by bariatric surgery in obese patients has led to a search to understand the influence of gut microorganisms and for additional gut-derived signals beyond the classical incretins, such as gastric inhibitory polypeptide (GIP) and GLP-1, that are known to stimulate insulin secretion in response to a meal. Such discoveries yield important, novel insights into the pathophysiology of the disease, and open the door to novel therapeutic approaches. Newly identified functions for nutrient receptors in the gut and other places and

how they may affect obesity and diabetes also need to be explored. Recently developed technologies are expected to yield numerous key advances in diabetes research over the next decade, particularly those used to manipulate and monitor neuronal pathways, combined with high-throughput "omics" approaches and functional neuroimaging.

Key Questions

- **What are the primary neurons in control of peripheral metabolism, and how are they integrated into a functional neurocircuit? To what extent are these pathways plastic and what factors can control their development and function?**

- **What are the relevant neuropeptides, transmitters, and intracellular signaling pathways in control of peripheral metabolism?**

- **Can functional neuroimaging reveal novel neural networks and pathways, and can it be a tool for personalized therapeutic approaches to treat metabolic disease?**

- **What is the full complement of gut-derived signals that participate in metabolic regulation, and what are their target tissues and modes of action?**

- **What specific physical alterations and humeral factors account for metabolic improvement following bariatric surgery?**

- **What can systems biology approaches reveal about gastric surgery in metabolic disorders or about the role of gut microorganisms in metabolic disease?**

- **Do intestinal microbiota play a role in diabetes and energy homeostasis? Is this role affected by diet and body weight?**

Future Directions

➤ **Characterize the functional neurocircuitry, including its adaptation and integrated regulation by hormones and nutrient components.**

Early studies in this area have identified primary neurons responsible for mediating the effect of individual peripheral mediators, such as insulin and leptin, as well as nutrients, such as glucose, fatty acids, and amino acids. However, developments in genetically encoded neuron tracing and remote control of defined neurons will lay the groundwork to define the functional neurocircuitry in control of peripheral metabolism. Moreover, defining the integrated control of numerous factors regulating the activity of key convergence nodes in this functional circuitry will lead to efficient modification of this regulatory system. Besides the analysis of acute regulatory mechanisms of this circuitry, understanding the genetic and epigenetic mechanisms of its development could help define novel interventions for impaired perinatal programming of this system, as there is growing evidence for plasticity in these metabolically important neurocircuits.

➤ **Apply a systems biology approach to understand metabolic improvements resulting from bariatric surgery.**

The integrated modeling of different sets of complex molecular data, including transcript profiling, proteomics, metabolomics, and multiplex assessments of simultaneous changes in circulating humoral factors, provides a promising approach to discover metabolic alterations and their control mechanisms. This is particularly true when a single intervention, such as bariatric surgery, evokes complex interacting changes in all of these systems. Large-scale data acquisition

of these parameters and subsequent mathematical modeling may define the key pathways responsible for metabolic improvement following bariatric surgery, and may define key regulatory nodes as potential targets for pharmacological intervention.

> **Use systems biology approaches to understand the effects of the gut microbiome on nutrient homeostasis and energy metabolism.**

Recent data suggest that the intestinal microflora can profoundly alter the efficiency of nutrient uptake from food, that the relative populations of bacteria are modulated by the obese state, and that bacteria from obese mice can result in increased fat storage when transplanted into a lean mouse. An interesting open question is whether these organisms play other roles in whole-body energy balance or the pathogenesis of diabetes—do they exert an impact on the relative concentrations of nutrients and the ability of the human body to store and burn them? Do gut-derived hormones and other signals from the gut or the bacteria themselves produce these effects, and how does this depend on an individual's complement of bacteria? Do they affect the immune system in such a way that protects from or encourages insulin resistance and diabetes? This rich and relatively understudied area deserves increased attention.

Defining the Subtypes of Type 2 Diabetes by Molecular Phenotyping

Given the accumulated evidence that type 2 diabetes can arise from any number of genetic, molecular, and environmental events, it stands to reason that different forms of the disease should respond best to therapies targeted to specific affected biological systems. Toward this end, a detailed description of

the genetic and metabolic staging of the disease in humans at the molecular and physiological level using high-throughput genetic, genomic, proteomic, and metabolomic technologies may allow researchers to define an individual's specific subtype of diabetes and stage of disease. The same technologies combined with appropriate bioinformatics and modeling will also significantly improve understanding of basic biology and disease mechanisms at a whole-body, systems biology level. Thus, it should be possible to integrate successfully these genetic, genomic, proteomic, and metabolomic data with information on functional outcomes and diseases in individuals.

Key Questions

- **What are the molecular phenotypes of different stages in diabetes pathogenesis? Can different diabetes subtypes be identified?**
- **Can easily measurable biomarkers for insulin resistance be defined in humans and rodents, and can models to predict development of the disease and its response to treatment be developed?**
- **Can molecular phenotyping strategies be successfully used to evaluate existing and novel therapies in clinical populations?**

Future Directions

> **Develop biomarkers for insulin resistance.**

Insulin resistance precedes and predicts the development of type 2 diabetes. Insulin resistance is also central to the metabolic syndrome and its various components—obesity, fatty liver disease, increased risk for atherosclerosis, reproductive dysfunction (especially polycystic ovarian disease), hypertension, Alzheimer's

disease, and even some forms of cancer. Nonetheless, at present, insulin resistance cannot be accurately measured at the clinical level other than by complex techniques such as the euglycemic, hyperinsulinemic clamp. A systems biology approach, with the various "omic" technologies, could be taken to identify new and more easily assessed biomarkers of insulin resistance. Such markers would serve as important research tools into disease pathogenesis, provide a biomarker for the pre-diabetic state, and be useful as tools to measure effects of therapy designed to improve insulin sensitivity in research studies and clinical practice.

➤ **Establish interdisciplinary research teams to study gene-environment interactions.**

To address important questions related to gene-environment interactions, basic experimental, clinical, genetic, and epidemiological studies will be required with interaction among multiple disciplines (e.g., endocrinology, nutrition, genetics, and genomics). Establishment of transdisciplinary educational and training opportunities in endocrinology, physiology, genetics, bioinformatics, computational and cell biology, and clinical research are needed to realize maximal benefit and impact from application of new technologies to diabetes research and treatment.

➤ **Develop a large, well-characterized clinical cohort of individuals ranging from normal metabolism to active type 2 diabetes.**

Establishing a diverse cohort large enough to include individuals with normal metabolism, individuals with various degrees of dysregulated metabolism (impairment of lipid and/or glucose homeostasis), and individuals with overt diabetes would allow researchers to apply the full complement of physiological and biochemical testing, imaging, and the various "omic"

technologies to create a platform for metabolic staging of type 2 diabetes and its disease progression. Progress toward this goal may be facilitated by pooling data from existing cohorts.

➤ **Develop tissue and serum banks at all stages of type 2 diabetes.**

Ready access to preassembled collections of tissue and plasma from well-characterized study participants will greatly accelerate the development of disease-specific metabolic fingerprints and allow researchers to compare results across technology platforms.

➤ **Employ molecular phenotyping for personalized medicine in diabetes.**

Molecular profiling technologies used along with computational and bioinformatics tools could result in new methods to stage and diagnose specific forms of diabetes. Such research could ultimately lead to the development of predictive algorithms and models for defining diabetes subtypes and directing therapy.

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

The onset and progression of type 2 diabetes affects multiple biological systems in the body, and varies from person to person—creating complex challenges for researchers and clinicians seeking to better understand, diagnose, prevent, and treat the disease. Now, new tools and technologies make it possible to assess the effect of type 2 diabetes on these systems, and vice versa. By gathering rich data sets in various aspects of diabetes biology, including gene-environment interactions, metabolism, inflammation, cell biology, the brain and digestive tract, and nutrient signaling, scientists will have a wealth of information with which to answer important questions specific to those areas and to understand the connections between them—thereby creating a more fully realized portrait of type 2 diabetes, its diversity, its harbingers, and its consequences. People with diabetes should benefit from this approach by seeing more rapid attainment of new therapies and of tailored approaches to prevention and treatment as this research moves forward.