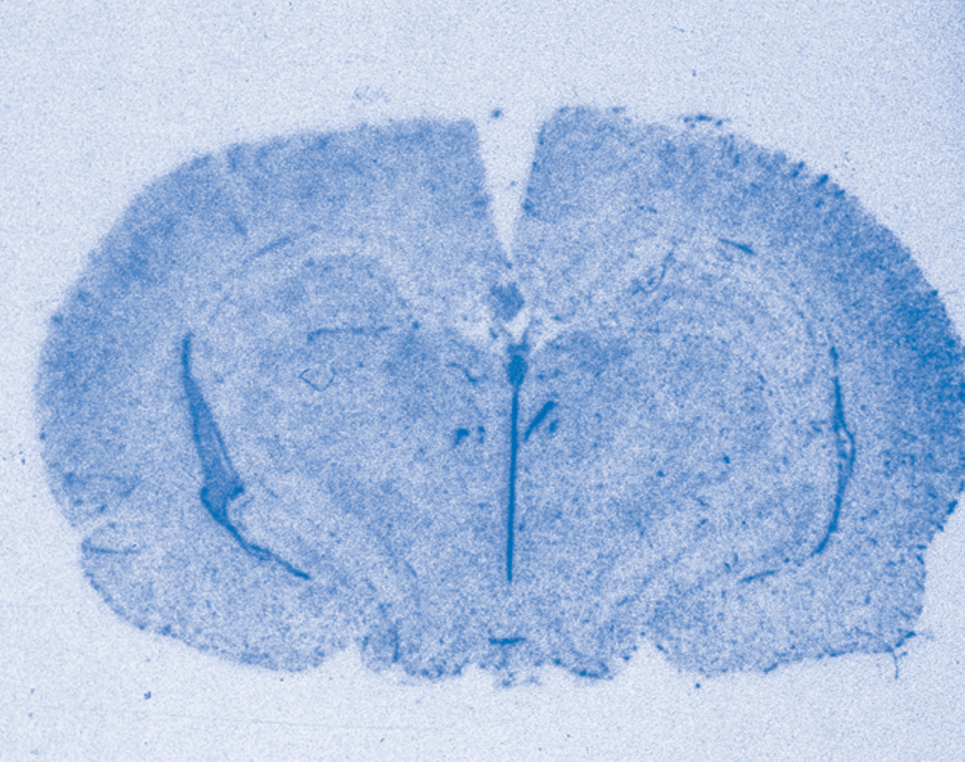

GOAL IV:

PREVENT OR REDUCE HYPOGLYCEMIA IN TYPE 1 DIABETES

Recent Scientific Advances

Brain and Peripheral Metabolic Sensing
Hypoglycemia-Associated Autonomic Failure (HAAF)
Adaptive/Maladaptive Brain Responses
Development of Animal Models of Hypoglycemia
Insulin Analogues and Glucose-Sensing Technology
Blood Glucose Awareness Training (BGAT)

Research Objectives and Strategies To Achieve Goals

Brain and Peripheral Nervous System Mechanisms of Hypoglycemia

- Define the Mechanisms and Modulators of Metabolic Sensing
- Elucidate Brain Alterations in Response to Hypoglycemia
- Develop New Strategies To Prevent or Reverse HAAF

Clinical Interventions To Prevent or Reduce Hypoglycemia

- Control Hypoglycemia Through Behavioral Therapies
- Close the Loop: Develop the Tools Required for an Artificial Pancreas

STRATEGIES TO PREVENT OR REDUCE HYPOGLYCEMIA IN TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

Although insulin therapy is the cornerstone of type 1 diabetes management and prevention of disease complications, excessive treatment with insulin can result in hypoglycemia. Too much insulin in the blood causes glucose levels to fall dangerously below a minimal threshold required to fuel the body's activities, particularly the brain. Even with newer forms of insulin that may decrease this risk, hypoglycemia remains an extremely serious, life-threatening concern.

The potential for hypoglycemic episodes has limited the use of intensive insulin therapy protocols that are known to reduce the risk of longer-term diabetic complications, such as eye, heart, and kidney disease. The immediate effects of hypoglycemia can be severe, including changes in cardiovascular and central nervous system function, cognitive impairment, increased risk for unintentional injury, coma, and death. Reducing the risk and impact of hypoglycemia would profoundly improve the quality of life for patients with type 1 diabetes.

Normally, a drop in blood glucose triggers the body's warning system to release stress hormones, including adrenaline, and to stimulate a part of the nervous system that raises glucose and results in symptoms such as shaking and sweating. The body also compensates with other "counter-regulation" defense measures, including the release of glucagon, a hormone that elevates blood glucose. However, in type 1 diabetes, glucagon release does not occur, and hypoglycemia warning signals often are not triggered due to an impaired adrenaline and nervous system response. The individual does not recognize and, therefore, cannot correct for the low blood glucose (hypoglycemia unawareness). Patients, especially children, are particularly vulnerable to hypoglycemia unawareness while they are asleep. Therefore, "nocturnal hypoglycemia" is a primary concern and the source of many anxious nights for parents of children with type 1 diabetes, who stay awake to check on the well-being of their children. The autonomic nervous system, which controls the activation of counter-regulation and warning-signal hormones that prevent hypoglycemia, becomes progressively impaired in type 1 diabetes, in large part possibly because of brain alterations that occur during each hypoglycemic episode. This vicious cycle of recurrent hypoglycemia is referred to as hypoglycemia-associated autonomic failure, or HAAF.

The widespread introduction and use of reliable, accurate, and relatively user-friendly self-monitoring glucose devices and portable insulin pumps have transformed the management of type 1 diabetes in the past two decades. Yet, because current therapy still requires painful finger sticks and injections, and in most cases still fails to achieve target glucose levels, it is critically important to develop noninvasive, continuous-monitoring, and improved insulin delivery technologies. In addition, designing a "closed-loop" delivery system or artificial pancreas—made by combining the glucose sensor and insulin pump—is the next important step in achieving close glucose control, until islet or beta cell replacement therapy becomes a viable option for patients with type 1 diabetes.

RECENT SCIENTIFIC ADVANCES

The phenomenon of hypoglycemia unawareness was originally described decades ago with the introduction of exogenous insulin. However, only recently have scientists begun to understand the complicated psychological, behavioral, and molecular mechanisms involved. This research, coupled with technological improvements in insulin therapy and behavioral interventions, is reducing the burden of hypoglycemia in type 1 diabetes.

Brain and Peripheral Metabolic Sensing: Maintenance of normal glucose balance (homeostasis) not only depends on the pancreas to release hormones in response to glucose levels, but also requires the communication of signals from all over the body to the brain, as well as from the brain itself. In the past decade, significant advances have revealed where and how the brain measures the body's metabolic status.

To measure glucose levels in the blood, cells with specialized molecular sensors—some of which are similar to those used by the pancreas—line vessels that lead to the liver and brain, as well as the gastrointestinal tract. These peripheral sensors are linked to groups of specialized glucose-sensing nerve cells (neurons) that are localized within a distributed, interconnected network within the brain, including the hypothalamus, forebrain, and hindbrain. To help the brain integrate different signals, some of these same brain neurons also respond to a variety of metabolic substrates (e.g., lactate, ketone bodies, fatty acids) and hormones (e.g., insulin, leptin, corticotropin releasing hormone), which are involved in the control of metabolism in the body. Identifying molecules and pathways for metabolic sensing may lead to targeted drug development to reduce hypoglycemia.

Hypoglycemia-Associated Autonomic Failure (HAAF): Why do most patients with type 1 diabetes suffer from recurrent bouts of hypoglycemia? The reasons became clear with the discovery that treatment-induced hypoglycemia reduces defenses against subsequent hypoglycemic episodes. Researchers have long recognized that patients with type 1 diabetes do not secrete glucagon in response to hypoglycemia, despite their ability to secrete glucagon under other circumstances. Recent findings suggest that a decrease in intraislet insulin is necessary for glucagon secretion, a response that does not occur in these patients. Identification and characterization of defects in the adrenaline warning system and autonomic response that commonly appear as insulin therapy is intensified (HAAF) have contributed to the understanding of hypoglycemia unawareness, and have laid the groundwork for future treatment.

Adaptive/Maladaptive Brain Responses: Therapies designed to protect the brain from injury due to hypoglycemia require a basic understanding of brain fuel usage and its adaptation to recurrent episodes of hypoglycemia. Recent progress has revealed how glucose and other fuels are transported into the brain despite a blood-brain barrier that blocks most molecules from entry. Surprisingly, new measurements show that glucose levels bathing the brain are only 25 percent of those in blood, which indicates that the brain's glucose supply is very tenuous, particularly during hypoglycemia. Recent studies in rodents suggest that glucose transport into the brain may be increased by prior exposure to hypoglycemia and that brain glycogen (starch) may serve as a short-term fuel reserve to partially protect the brain from injury. Studies in patients suggest that the brain may more efficiently use other (non-glucose) fuels to meet its energy needs. Ironically, while these mechanisms do partially protect the brain from being damaged by impending hypoglycemia, they attenuate the ability of patients to actually recognize and respond to hypoglycemia quickly (i.e., before dangerously low glucose levels impair brain function). With a variety of complex factors modifying cognitive outcomes in patients, it is not yet fully clear how type 1 diabetes and insulin treatment alter sensitivity of the brain to hypoglycemia.

Development of Animal Models of Hypoglycemia: The neural systems that sense and respond to hypoglycemia are localized in brain areas and peripheral organs not readily accessible for study in human beings. For this reason, the development of animal models has been critical for understanding how the brain detects and responds to single and repeated bouts of hypoglycemia. These models mimic many of the same neural, hormonal, and behavioral deficits seen in humans and are beginning to yield important new information about the body's adaptation to recurrent hypoglycemia.

Insulin Analogues and Glucose-Sensing Technology: Use of intensive insulin therapy to achieve near-normal averages for long-term blood glucose control delays the development of vascular complications of diabetes, but at the cost of a three-fold increase in the risk of severe hypoglycemia. The frequency and potential consequences of severe hypoglycemia are much greater in children than in adults. The development and widespread use of new forms of insulin have advanced the ability to provide more physiological insulin replacement. The insulin analogues, to some extent, have reduced the incidence of hypoglycemia and, coupled with new technologies available for blood glucose measurements, have made it easier for patients to manage their blood glucose. In addition, the recent introduction of continuous glucose monitoring systems to guide insulin replacement is potentially one of the most important recent advances in the treatment of type 1 diabetes because it opens the pathway to development of an artificial endocrine pancreas.

Blood Glucose Awareness Training (BGAT): Research has demonstrated that behaviors can be taught that can significantly reduce the occurrence and magnitude of hypoglycemia. BGAT is a psycho-educational program developed for patients with type 1 diabetes. It focuses on improving patients' recognition and management of extreme blood glucose levels. Analyses of the effects of BGAT have demonstrated dramatic benefits. BGAT was found to improve patients' ability to: detect both high and low blood glucose levels; judge whether they should increase or decrease their blood glucose levels; and judge whether or not they could

drive. The training program also resulted in reduced episodes of severe hypoglycemia and diabetic ketoacidosis; decreased numbers of automobile accidents; reduced fear of hypoglycemia; and improved quality of life. Because the fear of hypoglycemia is a severe limitation to the practice of intensive insulin therapy, this type of training not only can greatly benefit patients in the short-term, but also can lead to long-term benefits by permitting them to more effectively control their blood glucose levels to prevent the development of disease complications.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

The dysfunctions that lead to hypoglycemia derive from the effects of diabetes and insulin therapy on the brain. Therefore, to prevent or reduce hypoglycemia, research must focus on how the brain measures glucose levels, how it adapts to hypoglycemic events, and how neural pathways are involved in hypoglycemia unawareness and autonomic failure. Furthermore, a major research objective is to develop clinical interventions that enable patients to better control their insulin levels.

Brain and Peripheral Nervous System Mechanisms of Hypoglycemia

For the nervous system to recognize if blood glucose levels are too high or too low, specialized cells both within brain tissue and in other tissues, such as the gastrointestinal tract and pancreas, must be able to detect small changes in blood glucose. Metabolic sensing is a new and burgeoning area of basic research that is critical for the development of therapeutic interventions for the prevention and treatment of hypoglycemia. In addition to characterizing the specific cellular mechanisms involved in glucose sensing, scientists will need to understand how the neural responses to glucose levels are modulated by factors in the blood, such as hormones and metabolites, and how sensing mechanisms are integrated and signals are relayed to different areas of the brain and peripheral tissues. Localization of these brain areas and neural networks in humans can be achieved through improvements in the resolution and sensitivity of imaging technologies (see Goal VI).

Progress in hypoglycemia research depends on understanding not only brain and peripheral metabolic sensing, but also brain alterations in response to hypoglycemia and mechanisms underlying HAAF. HAAF occurs as a result of episodes of hypoglycemia that commonly occur during insulin therapy. These episodes suppress and lower the level of glucose at which subsequent counter-regulatory responses occur, particularly the release of the hormone adrenaline. Adrenaline is the critical defense against hypoglycemia for type 1 diabetes patients because they already lack natural insulin and glucagon responses to hypoglycemia. Furthermore, suppression of adrenaline and sympathetic nervous system responses reduces the symptoms of low blood glucose, such as increased heart rate, sweating, and a desire to eat. These warning symptoms alert patients to take corrective action.

HAAF can be reversed by as little as several weeks of scrupulous avoidance of hypoglycemia. Unfortunately, this is extremely difficult to accomplish in clinical practice without causing deteriorating glucose control—an undesirable consequence with respect to diabetes complications. Thus, it is of critical importance to define the currently unknown mechanisms responsible for HAAF, so that new clinical strategies could be developed to prevent, correct, or compensate for HAAF, while at the same time, improving blood glucose control.

Research Objective—Define the Mechanisms and Modulators of Metabolic Sensing:

- *Identify and elucidate the mechanisms involved in glucose sensing in the brain.*

Multiple mechanisms involved in sensing blood glucose concentrations are being discovered. The same enzyme that pancreatic islets use to measure glucose (glucokinase) may play a role in the brain. Recent evidence suggests that glucose sensors use chloride or ATP-sensitive potassium ion channels, which increase or decrease the electric state of brain cells by allowing charged ions to pass in response to binding a signaling molecule. The enzyme that muscle and other tissues use to sense fuel deficits (i.e., AMP-kinase) may also be used by the brain sensors.

Glucose-sensing mechanisms are also evident in peripheral tissues, including the pancreas, liver, carotid body, and gastrointestinal tract. Thus, further characterization of glucose-sensing tissues in the periphery may provide a model for understanding brain mechanisms. Identification of the molecular mechanisms involved in glucose sensing and how they are altered by diabetes, its treatment, and previous exposure

to hypoglycemia will facilitate the development of pharmacological agents targeted for hypoglycemia prevention.

- *Determine the hormonal and metabolic modulators involved in glucose sensing.*

Insulin, the hormone that causes hypoglycemia, may also alter the responsiveness of glucose-sensing mechanisms and, ultimately, the responses of the brain to hypoglycemia. Circulating peripheral insulin gains access to the brain and elicits changes in release of neuropeptides and neurotransmitters. Similarly, other metabolic hormones (e.g., leptin, corticotropin-releasing hormone), fuels (e.g., short- and medium-chain fatty acids), or nitric oxide have been suggested as modulators of glucose-sensing mechanisms. Finally, glucose sensing might be modulated by changes in the number of glucose transporters—molecules that recognize glucose outside the cell and bring it inside; or altered regulation of cellular receptors—molecules on the surface of cells that take up hormones and neurotransmitters so that they can do their work in the body.

In addition to nerve cells, the brain contains other types of specialized cells that may play a critical role during hypoglycemia. Specifically, glia (non-neuronal supporting cells within the brain) have the capacity to provide a source of energy to neurons during hypoglycemia by supplying both lactate (an energy-yielding breakdown product of glucose) and glycogen (a form of stored glucose arranged in long chains that can be broken down when needed). Future studies will need to determine if altered glucose sensing in diabetes or following hypoglycemia might be mediated by changes in glial function. In keeping with this possibility, glial-derived lactate has been reported to act as a signaling molecule in glucose-sensing neurons.

Research Objective—Elucidate Brain Alterations in Response to Hypoglycemia:

- *Determine alterations in brain metabolism and function induced by recurrent hypoglycemia.*

The brain appears to respond to the stress imposed by insulin-induced hypoglycemia by using mechanisms to ensure a continued supply of energy and protect the brain from damage. Varied scientific approaches are required to define how metabolism in the brain changes or adapts to reduce the insult and injury from recurrent episodes of hypoglycemia. A first step is to establish animal models of hypoglycemia in diabetes that replicate adaptive mechanisms of the brain. This resource will enable researchers to test therapeutic agents, isolate brain sections in culture, and apply newly developed gene array technologies directly to brain tissue, thereby facilitating identification of new protective mechanisms. Animal models would also be useful for protocols not ethically possible in humans, such as examining if the level of glucose control in diabetes modifies the brain's metabolic responses to recurrent hypoglycemia.

The relative importance of the mechanisms involved in the brain's attempt to protect itself from injury must be established so that, ultimately, they may be targeted for therapeutic intervention. Potential mechanisms to investigate include: increased transport of glucose into the brain; increased storage of glucose as glycogen; and alternative fuel utilization (e.g., lactate, fatty acids), as occurs normally during starvation. Approaches to these questions can be tested clinically with new state-of-the-art brain imaging technologies, particularly positron emission tomography (PET) and magnetic resonance spectroscopy (MRS).

Of significant clinical concern is the effect of recurrent hypoglycemia on cognitive function. While it is known that cognitive function is impaired by acute hypoglycemia, the impact of intensive insulin therapy and hypoglycemia unawareness on cognition is poorly understood, in part because of the limitations of standard cognitive testing procedures. This issue can be addressed clinically with sensitive tools, such as functional magnetic resonance imaging (fMRI). This technology allows scientists to directly image the local activity within patients' brains while they are performing cognitive tests under conditions of hypoglycemia. Finally, insulin itself could alter cognition. Thus, studies need to determine the contribution of insulin per se to the changes in cognition seen during insulin-induced hypoglycemia.

- *Prevent hypoglycemia-induced brain injury and promote protective adaptations.*

A major goal of uncovering both the adaptive and maladaptive mechanisms occurring in response to hypoglycemia is to develop therapies to reverse brain damage. Studies using state-of-the-art imaging technologies to assess brain function and metabolism could be performed in patients in whom hypoglycemic events are virtually eliminated, such as patients treated with an artificial pancreas or an islet transplant. These patients would be compared to type 1 diabetes patients with and without frequent episodes of hypoglycemia and hypoglycemia unawareness. Understanding the natural protective adaptations of the brain could lead to interventions to further promote protection from injury, as well as maintenance of cognitive performance during hypoglycemia. For example, if the brain protects itself by increasing its use of alternative

fuels, patients could take oral supplements to augment their supply of these fuels. If the brain burns glycogen during hypoglycemia, patients could take agents that promote glycogen storage.

▸ *Identify potential genes involved in individual susceptibility to hypoglycemia.*

Little is known about the potential influence of genetic risk factors in patients who suffer from frequent episodes of severe hypoglycemia. Angiotensin converting enzyme (ACE) may play a role because high ACE activity and the presence of the D allele of the ACE gene are known to predict a high rate of severe hypoglycemia in type 1 diabetes. However, further genetic screening of highly susceptible patients could help identify other genes and, potentially, prevention strategies.

Research Objective—Develop New Strategies To Prevent or Reverse HAAF:

▸ *Elucidate the mechanisms of HAAF.*

Given the lack of understanding of HAAF, prevention will require experiments in animal model systems before rational approaches can be developed to enhance hormone defense systems in diabetes. Animal models afford the opportunity, at the molecular level, to determine whether hypoglycemia itself alters the function of glucose-sensing nerves, and, if so, how this occurs. For example, is it due to a change in nerve cell signaling, metabolic function of the cells, circulating or brain-derived hormones, brain neurotransmission, or a combination of some of these factors?

Furthermore, it is important to develop animal models of HAAF that not only replicate the pathophysiology of diabetes in humans, but also are consistent among laboratories. This approach will facilitate the establishment of a central repository and/or resource listing for molecular probes, cell lines, and transgenic animals for further research studies on HAAF. A greater understanding of the putative molecular mediators leading to defective hormone responses in type 1 diabetes will provide the basis for testing unique therapies aimed at reversing HAAF. These ideas would initially be explored in animal models, then eventually translated to clinical drug trials to test if the responses to acute hypoglycemia are improved and hypoglycemic risk is reduced.

▸ *Identify the clinical consequences of HAAF.*

In humans, HAAF can be examined by applying newly improved imaging technologies, such as PET, fMRI, or MRS, to study the activation and integration of specific areas of the brain that trigger hormonal defenses against hypoglycemia. Localization of region-specific activation patterns in humans will depend on continued improvements in the sensitivity of imaging techniques. These imaging studies should be performed in conjunction with functional monitoring of neuroendocrine, metabolic, and peripheral nervous system responses, as well as cognitive and behavioral responses. Integrative, multidisciplinary studies of this nature are required to investigate the broad pathological consequences of HAAF in humans. For example, recent studies demonstrate that the sympathetic nervous system's responses to hypoglycemia are reduced during sleep. Therefore, patients with type 1 diabetes are less likely to be awakened by hypoglycemia. This sleep-related HAAF, in the context of imperfect insulin replacement, probably explains the high frequency of nocturnal hypoglycemia in type 1 diabetes. Further studies are required to directly assess the impact of prior hypoglycemia at night on the counter-regulatory and neural responses during both sleep and exercise.

▸ *Develop and test therapies to restore counter-regulation.*

The complete loss of glucagon response to hypoglycemia develops in nearly all type 1 diabetes patients within a few years of diagnosis, independent of HAAF. Undoubtedly, if the glucagon response to hypoglycemia could be restored, the problem of hypoglycemia would be greatly minimized. Current data suggest that the inability of patients to suppress their intraislet insulin secretion may, in part, explain the absent glucagon response. Therefore, mechanistic studies characterizing the glucagon defect at the whole organ, cellular, and molecular levels would have great potential benefit for the development of agents to overcome the glucagon defect, because the alpha cell works normally in type 1 diabetes patients at other times.

Clinical Interventions To Prevent or Reduce Hypoglycemia

The frequency of hypoglycemia in patients with diabetes depends on many factors, including patients' management of blood glucose levels, training and ability to recognize the circumstances that precede hypoglycemia, and other individual differences. Behavioral and clinical strategies can be tailored to the individual needs of patients to prevent or reduce hypoglycemic episodes.

Despite improvement in clinical management, treatment of type 1 diabetes with exogenous insulin replacement will not be optimal until there is feedback control of insulin delivery, accomplished either by beta cell replacement or by a mechanical, closed-loop insulin delivery system based on

continuous glucose monitors (CGMs). Children and adolescents are particularly vulnerable to hypoglycemia. Therefore, they are an ideal target population for closed-loop insulin delivery, because they are likely to receive the greatest benefit from it and are not appropriate candidates for islet transplants or other experimental approaches that currently involve lifelong immunosuppressive therapies.

The development of accurate and reliable CGMs is the first step toward closed-loop insulin delivery. In comparison to the mature home glucose meter technology that has benefited from 25 years of development, CGM technology is still in its infancy and needs further refinement. As has been demonstrated by the Diabetes Research in Children Network (DirecNet), the first generation devices approved by the FDA have major limitations.

Progress toward development of an artificial pancreas is likely to be a stepwise, iterative process. Several new and improved "real-time" CGM systems have recently been introduced, and may be followed by the development of algorithms that allow for appropriate insulin delivery via continuous delivery systems.

Research Objective—Control Hypoglycemia Through Behavioral Therapies:

- *Refine and link behavioral interventions and algorithms that predict risks of hypoglycemia.*

Hypoglycemia is not a homogenous phenomenon that affects every patient with diabetes in the same way; some patients are more vulnerable to morbidity and mortality. For example, recent research has shown that patients with recurrent driving mishaps have particular biological and behavioral characteristics (e.g., exhibiting enhanced insulin sensitivity, greater cognitive and motor decay with equivalent levels of hypoglycemia, and other characteristics such as being more likely to live alone). Clinical research that maps patients' behavioral traits could assist the development of algorithms that predict the risk of adverse hypoglycemic events. These models could be coupled with interventions that reduce the risk in identified patients (e.g., preventing driving mishaps). Behavioral research could help patients improve the recognition and prevention of hypoglycemia.

- *Evaluate behavioral approaches to preventing nocturnal hypoglycemia.*

Nocturnal hypoglycemia remains a significant clinical problem and source of concern for parents of children with type 1 diabetes. A practical aid to them in helping their children maintain normal blood glucose levels would be a systematic evaluation of the impact of the dietary composition of evening meals and snacks to reduce the incidence of nocturnal hypoglycemic events and to improve subsequent morning glucose values.

Research Objective—Close the Loop: Develop the Tools Required for an Artificial Pancreas:

- *Optimize use of continuous glucose monitors.*

Currently approved CGMs are limited in their ability to reliably detect low blood glucose. Reliable identification of hypoglycemia in the home setting would minimize the risks associated with hypoglycemic events and permit the evaluation of factors, such as exercise and diet, that influence the risk of hypoglycemia, especially during the night. Once reliable sensors are developed, it will be possible to conduct clinical trials of real-time glucose sensor monitoring with outcome measures, including reducing the risk of hypoglycemia, lowering HbA1c levels, and enhancing counter-regulatory hormone responses using insulin pumps or basal/bolus injection regimens. As part of these investigations, guidelines could be developed on how to use glucose sensor data to optimize glucose control and minimize the risk of severe hypoglycemia.

- *Develop algorithms needed to link glucose monitors with insulin delivery.*

The development of computerized algorithms that automatically vary insulin delivery rates based on glucose sensor data is essential to providing adequate control of postprandial hyperglycemia and eliminating the risk of hypoglycemia. Further studies would test how robust such systems are over time and under a variety of real-life conditions. Once reliable sensors are developed and appropriate algorithms are established, their integration into a closed-loop system would be feasible. The development of closed-loop insulin delivery would initially start with external, minimally invasive, subcutaneous, short-term sensors and external pumps, and would lead ultimately to fully implantable, long-term systems. This progress will require cooperation among the NIH, industry, and private foundations. Moreover, it will require enhanced collaborations among diabetes specialists, islet physiologists, bioengineers, and the computational biology/informatics community.