Evaluation of Counter-regulatory Hormone Responses during Hypoglycemia and the Accuracy of Continuous Glucose Monitors in Children with T1DM

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CHAPTER 1
INTRODUCTION

1.1 Introduction and Rationale

1.1.1 Hormone Responses during Hypoglycemia

Hypoglycemia is a life-threatening side effect of intensive insulin therapy in patients with Type 1 diabetes (1, 2) This is especially the case in children and adolescents and it is observed as an acute problem in very young children.(3) In all aspects of the daily care of children with diabetes the degree of burden is higher in younger children than in older children, adolescents or adults. The recent increase in the number of very young children diagnosed with diabetes and the ever-present dilemma between good glucose control and the risk of hypoglycemia in these children have brought to light the problem of detection of hypoglycemia and the factors affecting the response to hypoglycemia in these children.(4) The use of new methods of continuous glucose monitoring has revealed multiple instances of asymptomatic hypoglycemia both nocturnal and during awakening. This phenomenon, although present in all children and adolescents, is much more frequent in younger children. As a result, these very young children have a higher frequency of seizures, nocturnal or otherwise, which may result in possible neurocognitive deficits at a later age.(5)

In children with Type 1 diabetes, the phenomena associated with hypoglycemia such as counter regulatory hormone responses and autonomic responses to hypoglycemia have not been evaluated as extensively as in adults.(6) In adults, extensive studies have addressed the effect of diabetes and other factors on counter-regulatory hormone and symptomatic responses to hypoglycemia.(1, 2) These studies were made possible by the development of insulin infusion protocols and the hypoglycemic clamp technique to provide a controlled, safe, and reproducible hypoglycemic stimulus in different groups of subjects or in the same subjects under different conditions. Using these techniques, it has been shown that long diabetes duration, intensive insulin treatment, exercise, sleep and antecedent hypoglycemia reduce adrenomedullary (plasma epinephrine), central sympathetic (plasma norepinephrine), and symptomatic response to hypoglycemia.(7) In most of these studies, reductions in sympathetic responses were related to a lowering of the plasma glucose level that was required to stimulate a rise in plasma catecholamine levels. In contrast, plasma epinephrine responses to hypoglycemia are increased in older children and adolescents with or without diabetes compared to corresponding responses in adults with or without diabetes (8) due to an upward shift in the plasma glucose threshold for release of catecholamines.(9)

In addition, studies in adults with Type 1 diabetes have addressed the effect of duration of diabetes as well as recent antecedent hypoglycemia on glucose counter-regulatory hormones response and autonomic response manifested as hypoglycemic symptoms.(10) In a number of elegant studies in adults with diabetes and normal subjects, several groups have experimentally induced hypoglycemia and measured the responses to hypoglycemia on days following normoglycemia or following another episode of hypoglycemia. There is consensus that antecedent hypoglycemia reduces adrenomedullary epinephrine, sympathetic neural nerepinephrine, neurogenic symptoms and glucagon on subsequent hypoglycemia, whereas Growth hormone and cortisol responses have been found to be unaltered in normal subjects.(11, 12) In addition exercise and the counter-regulatory hormone response to antecedent exercise have been shown to reduce epinephrine and growth hormone response to subsequent (next few days) hypoglycemia while cortisol, norepinephrine, glucagon and neurogenic symptoms were not affected.(13)

The ability to detect hypoglycemia depends on several systems that encompass the spectrum of neurocognitive maturity and level of hormonal counter-regulatory response maturity.(14) It is not

known if young children with diabetes have an intact counter-regulatory hormone response. In newborn infants it has been shown that a robust counter-regulatory hormone response to hypoglycemia can be elicited but sympathomimetic symptoms are usually lacking.(15)

The question of whether autonomic symptoms and counter-regulatory hormone response to hypoglycemia is dependent on age or developmental stage or is impaired by diabetes duration or frequency of antecedent hypoglycemia in children has not been examined adequately. Children have several physiologic factors that may affect response to hypoglycemia, such as developmental and maturity stage as well as hormonal and pubertal stage. Studies to date that have tried to address this issue in children have concluded that there was no difference noted in the different stages of development but the youngest children included in this study were 9 years old.(16) In addition in a different study examining the counter regulatory response to nocturnal hypoglycemia in prepubertal children, a lower hormonal response was only partially responsible for the prolonged nocturnal hypoglycemia observed in these 6-13 year old children.(17)

In comparison to children without diabetes, counter regulatory responses were lower in children with diabetes (mean age of 14 years for both groups) but autonomic symptom recognition was similar. Despite the lower counter regulatory hormone response in children with diabetes, the glycemic threshold for adrenaline to increase higher than 2 SD above basal was 63$\pm$3 mg/dL in these children.(18) In adolescent children with diabetes epinephrine responses during sleep were lower than in normal children (19) but in a controlled hypoglycemia setting with an insulin clamp, children with and without diabetes had higher epinephrine responses than adults.(8) In a study of insulin induced hypoglycemia (glucose <60mg/dL) adolescents with diabetes had a lesser catecholamine response than matched controls and this response was even lower in intensively treated youth with diabetes. Furthermore this study demonstrated that nadir glucose level is the important determinant of counter regulatory hormone response and not the rate of glucose decrease to the nadir glucose level.(20)

Despite the clinical importance of avoiding hypoglycemia in very young children with T1DM, no studies have examined and compared the adequacy of counterregulatory hormone defense mechanisms in very young versus older children with T1DM. This may be related to practical problems with carrying complex insulin infusion and glucose clamping procedures in infants and toddlers. Nevertheless, it is likely that the ability to detect and respond to hypoglycemia is reduced in very young patients. Moreover, parents of young children with T1DM often anecdotally report absence of any signs and symptoms of hypoglycemia, even in the face of plasma glucose levels < 30 mg/dl. In a study of six preschool age children, insulin induced hypoglycemia resulted in normal epinephrine, norepinephrine and growth hormone response but no glucagon response.(21) In this study intravenous insulin lowered glucose levels from 80 to 40mg/dL over one hour and counter regulatory hormone levels were only measured at baseline and at nadir glucose.

The present study is being undertaken to compare counterregulatory hormone responses to a mild and gradual reduction in plasma glucose in young children with T1DM versus responses in adolescents. The studies will be performed under the close supervision of the professional staff of each DirecNet center and frequent bedside monitoring of plasma glucose concentrations will ensure that clinically significant hypoglycemia is prevented from developing. All subjects will be admitted to the CRC and have an IV line for blood sampling inserted on the evening prior to study to reduce stress on the morning of the study. The study procedure will be simplified and made less invasive in comparison to a clamp or standard insulin infusion study (i.e. only the one IV for blood sampling will be needed) by limiting enrollment to insulin pump-treated subjects who will have their basal rates modestly increased to produce the hypoglycemic stimulus. Monsod and collegues used the

same procedure of increasing the basal insulin infusion dose to induce a gradual fall in plasma glucose in youth with type 1 diabetes in a study that compared the ability of injections of glucagon and epinephrine to treat mild hypoglycemia. (22) It is particularly important to note that once the blood glucose level falls below 60 mg/dl, a blood sample will be obtained and hypoglycemia will then be immediately corrected by intravenous administration of exogenous glucose. In our recent DirecNet study,(23) ~25% of children and adolescents had plasma glucose levels below 60 mg/dl during a typical night and this rose to ~50% of subjects when there was antecedent exercise in the late afternoon. Moreover, the frequency of mild as well as severe hypoglycemia is substantially higher in pre-school children than in children and adolescents. This safe and rigorously designed study will provide important new information regarding the role of inadequate counter-regulation on the increased risk of hypoglycemia in very young children with T1DM.

1.1.2 Evaluation of Continuous Glucose Monitoring Systems

Real-time continuous glucose sensing systems offer the potential to markedly lower the risk of hypoglycemia in youth with T1DM. However, the DirecNet inpatient accuracy study demonstrated that the first generation of these devices was inaccurate when blood glucose was lowered to less than 70 mg/dl. In that study, children with T1DM between 3-17 years of age were admitted to the CRC for approximately 26 hours during which they wore 1-2 Medtronic MiniMed CGMS and 1-2 Cygnus GlucoWatch G2 Biographer continuous glucose monitors. In every subject in that study, blood samples were obtained every 30-60 minutes from an indwelling intravenous catheter for measurement of reference plasma glucose levels in the DirecNet Central Laboratory. The Guardian-RT continuous glucose monitoring systems is a real-time continuous glucose monitor that has considerable promise for use in children with diabetes. Therefore, a secondary aim of this study is to obtain very important data regarding the accuracy of this system during hypoglycemia in young children, as well as adolescents.

The Guardian-RT has been approved by the FDA for detecting trends and tracking patterns in adults (18 and older) and are indicated for adjunctive rather than replacement of standard home glucose monitoring devices. The sensor has been approved by the FDA for use for up to 72 hours but can function for a longer period of time.

1.1.2.1 Background on the Guardian-RT

The Guardian-RT was developed and is distributed by Medtronic Minimed. This sensor uses a glucose oxidase based electrochemical sensor which generates 2 electrons for each glucose molecule oxidized. The current generated from measuring glucose is called the ISIG (Input SIGnal). The Guardian-RT system is designed to measure blood glucose levels in a range of 40-400 mg/dl. The sensor is inserted subcutaneously and measures interstitial glucose. When functioning properly, the Guardian-RT acquires glucose values every 10 seconds and these values are averaged in the monitor to provide a reading every 5 minutes (or 288 readings a day) which is transmitted wirelessly to a receiver that can be kept up to 6 feet from the transmitter. Each sensor is designed to measure readings for up to 72 hours.

The Guardian-RT has alarms for hypoglycemia and hyperglycemia that can be adjusted by the user. Subjects can enter events, such as when they took insulin, ate, or exercised. The sensor requires at least 2 capillary glucose readings each day (every 12 hours) to validate sensor function and allow for development of a calibration equation. These calibration measurements are performed with a home glucose meter, and calibration is dependent upon the subject entering glucose values correctly into the sensor.

1.2 Study Objectives

The primary objective of this study will be to compare the glucose level at which counter-regulatory hormone responses occur during hypoglycemia in young children with diabetes, with the glucose level counter regulatory hormone responses that occur in older children with diabetes. We hypothesize that the children in the younger age group will not have a counterregulatory response until a lower glucose level is reached compared with children in the older age group.

Secondary objectives will be:

1 To assess signs and symptoms at different glucose levels of hypoglycemia in younger children and compare those with the older children. Furthermore, symptoms will be compared to counter regulatory hormone levels in the two age groups. Symptoms will be assessed using physiologic data and using an age-appropriate questionnaire that would be completed during the test by the subject (where appropriate) and by a parent.

2 To assess whether there is a difference in counter-regulatory hormone response in each age group, among those who had at least 2-3 episodes of hypoglycemia per day or night prior to the study compared with those who had an occasional or no episodes of hypoglycemia prior to the test. Subjects will wear a Guardian RT for 6 days ($\pm$1 day) prior to the test to assess episodes of hypoglycemia.

3 To examine the accuracy of the Guardian-RT during hypoglycemia in children with type 1 diabetes.

1.3 Synopsis of Study Protocol

Sample Size: 50 Subjects

Summary of Protocol

1. Informed consent is obtained from eligible subjects (age 3 to $\leq$7 or 12 to <18 years, T1D for $\geq$1 year, insulin pump being used).
2. On the day of enrollment a hemoglobin A1c is obtained and instructions are given for use of the Guardian RT. The study personnel will supervise the subject or parent inserting the sensor in the clinic. The subject will be instructed to complete at least four glucose measurements a day using the study HGM. Instructions will also be given for response to Guardian RT alarms prior to the CRC admission.
3. The subject will return for an 18-hour overnight CRC admission approximately 6 days ($\pm$ 1 day) after the enrollment visit.
    - Subjects will continue using the Guardian RT sensor inserted prior to the admission.
    - For subjects of sufficient size to accommodate additional devices, a second Guardian-RT sensor will be inserted. An intravenous catheter will be inserted for reference measurements (glucose, epinephrine, norepinephrine, cortisol, glucagon and GH), which will be drawn during the subcutaneous insulin infusion test the following morning to send to a central laboratory.
    - For subjects of sufficient weight (subjects $\geq$14.9kg at reinfusion centers and $\geq$26.3kg at discard centers) to accommodate the volume of blood required, blood glucose measurements will be made every 30 minutes during the admission to allow for assessment of the accuracy of the Guardian-RT.

- For subjects of sufficient weight to accommodate the volume of blood required, blood glucose measurements will be made every 15 minutes for two hours after dinner. This will allow for assessment of the accuracy of the Guardian-RT in detecting change during a period of rising blood glucose.
- At approximately 8:00 a.m. the subcutaneous insulin infusion test will start.
    - Prior to starting the test, a HypoMon® may be placed around the subject's chest using an adjustable strap.
    - The glucose concentration as measured by the study HGM must be $\geq$110 mg/dL to start the test.
    - At the start of the test, the basal insulin rate will be increased by approximately 25-50% to provide a gradual decline in blood glucose. A small priming bolus dose of insulin equal to approximately one hour of the subject's usual basal dose may also be given at the discretion of the investigator in addition to the 25-50% increase in the basal insulin.
    - The basal insulin rate may be increased an additional amount and additional bolus insulin doses may be given at the discretion of the investigator in order to get a gradual decline in the glucose concentration.
    - Blood samples will be collected for the laboratory and glucose will be checked with the study HGM with venous blood every 15 minutes until the glucose level reaches 100 mg/dL. Thereafter, the study HGM will be used to check the glucose levels with venous blood every 5-10 minutes depending on the rate of fall of the glucose level until the end of the study.
    - Blood samples will be collected for laboratory determination of hormone concentrations at baseline (before increasing the insulin infusion) and when the glucose levels are <90, <80, <70, and <60 mg/dL.
    - Subjects (or parent if appropriate) will be asked questions regarding symptoms of hypoglycemia each time the glucose level is checked with the study HGM.
    - Once the endpoint is reached (the first time the glucose is <60 mg/dL using the study HGM), the basal rate will be returned to normal, the subjects will be treated with intravenous glucose and breakfast will be provided.

4. Prior to discharge, the sensors will be removed and downloaded and the subject's insulin pump will be downloaded if possible.

CHAPTER 2
SUBJECT ELIGIBILITY AND ENROLLMENT

2.1 Study Population

Approximately 50 subjects will be enrolled in this study at five clinical centers with approximately 10 enrolled at each center.

Enrollment will include approximately 25 subjects in each of the age groups of 3.0 to ≤7.0 years old and 12.0 to <18.0 years old.

Subjects will include both males and females and an enrollment goal will be to achieve an approximately equal sex distribution in each age group.

A goal of recruitment will be to enroll approximately 10% minorities.

2.2 Eligibility and Exclusion Criteria

2.2.1 Eligibility

To be eligible for the study, all subjects must meet the following criteria:

1) Clinical diagnosis of type 1 diabetes and using daily insulin therapy for at least one year
   The diagnosis of type 1 diabetes is based on the investigator's judgment; C peptide level and antibody determinations are not needed.
2) Age 3.0 to ≤7.0 years or 12.0 to <18.0 years
3) Weight ≥12.8 kg (28.2 lbs) for reinfusion centers (centers that employ reinfusion of blood drawn to clear the dead space in intravenous catheters) and ≥17.4 kg (38.3 lbs) for discard centers (centers that discard the blood drawn to clear the dead space)
4) Hemoglobin A1c ≤10.0%
5) Subject currently uses an insulin pump
6) Parent/guardian and subject understand the study protocol and agree to comply with it
7) Informed Consent Form signed by the parent/guardian and Child Assent Form signed by the subject

2.2.2 Exclusion

Subjects who meet any of the following criteria are not eligible for the study:

1) The presence of a significant medical disorder or use of any medication that in the judgment of the investigator will affect the wearing of the sensors or the completion of any aspect of the protocol.
   - *Adequately treated thyroid disease and celiac disease do not exclude subjects from enrollment*
2) A severe hypoglycemic event resulting in seizure or loss of consciousness in the last month
3) Use of systemic or inhaled corticosteroids in the last month
4) Cystic fibrosis

2.3 Patient Enrollment and Baseline Data Collection

Potential subjects will be evaluated for study eligibility through the elicitation of a medical history and performance of a physical examination by a study investigator.

2.3.1 Historical Information and Physical Exam

A history will be elicited from the subject and parent and extracted from available medical records with regard to the subject's diabetes history and current diabetes management. A standard physical exam (including vital signs and height and weight measurements) will be performed by the study investigator or his or her designee (a pediatric endocrinologist, pediatric endocrine fellow, or a pediatric endocrine nurse practitioner).

2.3.2 Informed Consent

For eligible subjects, the study will be discussed with the subject and parent/legal guardian (referred to subsequently as 'parent'). The parent will be provided with the Informed Consent Form to read and will be given the opportunity to ask questions. Subjects of appropriate age will either be given the Child Assent Form to read or it will be read to the child. If the parent and child agree to participate, the Informed Consent Form and Child Assent Form will be signed. A copy of the consent form will be provided to the subject and his/her parent and another copy will be added to the subject's clinic chart.

Written informed consent must be obtained from the parent or guardian prior to performing any study-specific procedures that are not part of the subject's routine care.

2.3.2.1 Authorization Procedures

As part of the informed consent process, each subject will be asked to sign an authorization for release of personal information. The investigator, or his or her designee, will review what study specific information will be collected and to whom that information will be disclosed. After speaking with the subject and their parent, questions will be answered about the details regarding authorization.

2.3.2.2 Special Consent Issues

The study population for this study includes children and adolescents. The consent form and study procedures will be discussed with each subject at a level in which they can understand. The study staff will ask questions of each subject to assess the autonomy and understanding of the study. Each subject will be asked to sign an assent form, if appropriate for the subject's age. Additionally, the parent(s) and/or guardian(s) of each subject will be asked to sign the consent form. They will be given the opportunity to ask questions throughout the study on all study related procedures.

2.3.3 Hemoglobin A1c Determination

The DCA 2000 will be used for baseline measurement of hemoglobin A1c.

2.3.4 Instructions for Home Procedures

Each subject will be provided with a Guardian RT and sensors. The subject and parent/guardian will be instructed in the use of the device including calibration of the device using the study HGM. In order to assess accuracy at different points in the lifespan of the sensor, approximately 50% of the subjects will be asked to insert a new Guardian RTS sensor four days before the scheduled CRC admission and approximately 50% will be asked to insert two days before the scheduled CRC admission.

The subjects will be instructed to perform at least 4 blood glucose measurements per day prior to the CRC admission using the study HGM. The measurements will be performed prior to each meal and before bed. Additional HGM measurements may also be required for calibration of the device every 12 hours.

CHAPTER 3
SUBCUTANEOUS INSULIN INFUSION TEST

3.1 CRC Admission

About 6 days (±1 day) following the enrollment visit, subjects will have an inpatient CRC admission of approximately 18 hours. Subjects will be admitted at approximately 3:00 PM to allow sufficient time to calibrate the sensors before dinner is provided.

- Areas where a Guardian RT sensor was worn during the first week will be assessed by study personnel for any skin irritation.
- The Guardian RT, HGM, and pump data from the previous week will be reviewed and changes will be made to diabetes management as needed.
- Subjects will continue using the Guardian RT sensor last inserted at home. If the sensor is not functioning properly, a new sensor will be inserted.
- For subjects of sufficient size, an additional Guardian-RT sensor will be inserted and calibrated approximately two hours later. An intravenous catheter will be inserted in an arm vein for collection of blood samples during the admission. The area where the catheter will be inserted may be numbed with Elamax or EMLA cream prior to catheter insertion.

3.2 Pre-Subcutaneous Insulin Infusion Test

3.2.1 Blood Glucose Measurements

Blood glucose measurements will be made using the study HGM at bedtime, 12 a.m., 3 a.m., 6 a.m., and 7 a.m. to ensure the blood glucose level will be above 110 mg/dL at the time of the start of the test.

- Oral carbohydrates or a correction dose of insulin may be given if the subject's glucose level is too low or too high.
    - Glucose level will be repeated 30 min later if treatment was given for low glucose or 90 minutes later if a bolus insulin dose was given for high glucose.
    - Glucose will be considered to be low if less than 80 and will be treated with 15 grams of carbohydrates.
    - Glucose will be considered to be high if above 200mg/dl at midnight, 170mg/dl at 3 a.m. and 150 mg/dl at 6 a.m. A correction dose of insulin may be given for high glucose at the discretion of the investigator.

3.3 Subcutaneous Insulin Infusion Test

Subjects will have a subcutaneous insulin infusion test performed in the morning following the overnight admission at approximately 8:00 a.m.

Prior to starting the test, a HypoMon® (AiMedics Ltd, Sydney, Australia) may be placed around the subject's chest using an adjustable strap. This monitor, which is about the size of a remote control, uses wireless technology to pick up skin impedance and ECG signals from a thin belt strapped around the subject's chest.

The glucose concentration as measured by the study HGM must be ≥110 mg/dL to start the test.

At the start of the test, the basal insulin rate will be increased by approximately 25-50% to provide a gradual decline in blood glucose. A small priming bolus dose of insulin equal to approximately one hour of the subject's usual basal dose may also be given at the discretion of the investigator in addition to the 25-50% increase in the basal insulin.

The basal insulin rate may be increased an additional amount and additional bolus insulin doses may be given at the discretion of the investigator in order to get a gradual decline in the glucose concentration.

The test is expected to last approximately 1.5 to 2 hours. If the subject has not reached the endpoint (<60mg/dL) within 3 hours, the test will be stopped at the discretion of the investigator.

3.3.1 Blood Glucose Measurements

The clinical centers either will use reinfusion of blood or will discard blood with each blood draw, depending on the standard practice at each center's CRC. The blood draws will be performed by the method in standard use at the CRC. The blood samples will be sent to a central lab.

Glucose concentrations will be checked on the study HGM every 15 minutes with venous blood until the glucose level reaches ≤100 mg/dL. Thereafter, the study HGM will be used to check the glucose levels in 5-10 minute intervals with venous blood depending on the rate of fall of the glucose level until the end of the study. Subjects and parents will be masked to the results of the glucose testing during the subcutaneous insulin infusion test in an attempt to eliminate bias when answering questions regarding symptoms of hypoglycemia.

- Blood samples will be collected for laboratory determination of glucose and hormone concentrations at baseline when the subject's glucose level is between 95 and 110 mg/dL (in duplicate) and when the glucose levels are <90, <80, <70, and <60 mg/dL.
- Parents (and subject if appropriate) will be asked questions regarding symptoms of hypoglycemia each time the glucose level is checked with the study HGM.
- Once the endpoint is reached (the first time the glucose is <60 mg/dL using the study HGM), the basal rate will be returned to normal, the subjects will be treated with intravenous glucose and breakfast will be provided. An additional blood sample will be collected for laboratory determination of glucose and hormone concentrations 15 minutes following the treatment with intravenous glucose.

3.3.2 Blood Samples for Hormone Concentrations

Blood samples will be collected for epinephrine, norepinephrine, cortisol, glucagon and GH at baseline when the subject's glucose level is between 95 and 110 mg/dL (in duplicate) and when the glucose levels are <90, <80, <70, and <60 mg/dL. A final sample will be collected 15 minutes following the treatment with intravenous glucose when the subjects glucose level is <60 mg/dL.

As permitted by weight, additional samples may be collected for possible later analysis of pancreatic polypeptides, IGF BP1, insulin and others each time a sample is collected for glucose and hormone concentrations.

All samples collected for hormones will be collected, frozen, and shipped to the central laboratory for storage. A determination will be made once the primary outcome is known regarding which samples to process.

3.4 Post-Subcutaneous Insulin Infusion Test

Once the study endpoint is reached (the first time the glucose is <60 mg/dL using the study HGM), the basal rate will be returned to normal and the subjects will be treated with intravenous glucose. An additional blood sample will be collected for laboratory determination of glucose and hormone concentrations 15 minutes following the treatment with intravenous glucose. Subjects will then be given breakfast and discharged. Prior to discharge, the sensor(s) will be removed and downloaded. The subject's insulin pump will be downloaded if possible.

3.5 Daily Activities

Subjects will be permitted to perform their usual indoor activities during the hospitalization, except during the subcutaneous insulin infusion test where they will be restricted to bed.

3.6 Diet

The prescribed diet will be at the discretion of the investigator.

CHAPTER 4
EVALUATION OF CONTINUOUS GLUCOSE MONITORS

4.1 Overview

The accuracy of the Guardian-RT will be evaluated during the inpatient phase.

4.2 Inpatient Accuracy Evaluation

The inpatient stay is described in the prior chapter.

At the start of the admission, the following will be done:

- Areas where a Guardian RT sensor was worn during the previous week will be assessed for skin reactions.
- Subjects will continue using the Guardian RT sensor inserted prior to the admission.
    - If the sensor is not functioning at the time of the admission, a new sensor will be inserted.
- For subjects of sufficient size, a second Guardian-RT will be inserted. The time of placement and the placement site of each sensor will be recorded.

For subjects of sufficient weight to accommodate the blood volume requirements, reference glucose measurements will be made every 30 minutes during the CRC admission for evaluation of the accuracy of the Guardian-RT. The blood draws will be timed to be on the half-hour.

During the insulin-induced hypoglycemia test, blood glucose measurements will be made as described in section 3.3.

For subjects of appropriate weight to accommodate the volume of blood required for testing, the Guardian RT will be assessed following a physiologic rise in blood glucose after dinner.

Before starting the post-meal glucose measurements, a blood glucose level will be obtained using the study HGM. The subject's usual meal dose (the dose for the carbohydrates to be consumed) will be given, and a correction dose will be given if needed. Reference glucose levels will be obtained at baseline (when the subject finishes the meal) and every 15 minutes for 120 minutes.

4.2.1 Volume of Blood Draws

Each blood draw will require a blood volume of approximately 1.3 ml per blood draw at the "discard" centers and 0.3 ml per blood draw at the "reinfusion" centers. At the "discard" centers, the maximum number of blood draws based on the subject's weight will be calculated at the time of admission so that the maximum blood volume drawn will not exceed 5% (at reinfusion centers, the maximum blood volume drawn will not approach 5%). Section 5.5.6 provides further details on the blood volume requirements.

4.2.2 Quality Control Specimens

Approximately 5% of the reference blood samples collected for evaluation of the Guardian-RT will be collected in duplicate to send to the central lab for quality control purposes.

4.2.3 Sensor Considerations during Inpatient CRC Admission

4.2.3.1 Sensor Calibrations

If a calibration of the Guardian-RT is required during the admission, it will be performed by study/CRC personnel using a fingerstick sample.

4.2.3.2 Sensor Failure

If a sensor fails with less than 2 hours remaining until the start of the subcutaneous insulin infusion test, it will not be replaced.

4.2.3.3 Sensor Downloading

Prior to discharge from the CRC, all sensors will be removed and downloaded.

CHAPTER 5
ADVERSE EVENTS

5.1 Definitions

An adverse event is any untoward medical occurrence in a study subject, irrespective of whether or not the event is considered treatment or device-related.

5.2 Recording of Adverse Events

Throughout the course of the study, all efforts will be made to remain alert to possible adverse events or untoward findings. The first concern will be the safety of the subject, and appropriate medical intervention will be made.

The investigator will elicit reports of adverse events from the subject at each visit and complete all adverse event forms online. Each adverse event form is reviewed by the Coordinating Center to verify the coding and the reporting that is required.

The study investigator will assess the relationship of any adverse event to be related or unrelated by determining if there is a reasonable possibility that the adverse event may have been caused by the study device or study procedures.

The intensity of adverse events will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe. It is emphasized that the term severe is a measure of intensity: thus a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

Definitions of relationship and intensity are listed on the website data entry form.

Adverse events that continue after the subject's discontinuation or completion of the study will be followed until their medical outcome is determined or until no further change in the condition is expected.

5.3 Reporting Serious or Unexpected Adverse Events

A serious adverse event is any untoward occurrence that:

- Results in death
- Is life-threatening; (a non life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in significant disability/incapacity
- Is a congenital anomaly/birth defect

An *Unanticipated Adverse Device Event* is defined as an adverse event caused by, or associated with, a device, if that effect or problem was not previously identified in nature, severity, or degree of incidence.

Serious or unexpected adverse events must be reported to the Coordinating Center immediately via completion of a serious adverse event form.

The Coordinating Center will notify all participating investigators of any adverse event that is both serious and unexpected. Notification will be made within 10 days after the Coordinating Center becomes aware of the event.

Each principal investigator is responsible for informing his/her IRB of serious study-related adverse events and abiding by any other reporting requirements specific to their IRB.

5.4 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board will approve the protocol prior to its initiation and will be informed of all serious adverse events and any unanticipated adverse device events that occur during the study.

5.5 Risks and Discomforts

The FDA has determined that this protocol will require an Investigational Device Exemption (IDE). Adequate safeguards are in place to ensure that exposure to the risks associated with study participation are minimized to the extent possible.

The planned study also includes a Subcutaneous Insulin Infusion Test on the morning following the overnight admission during which slightly increased insulin doses will be used to achieve gradually falling blood glucose levels while serial blood samples are obtained for measurement of various counter-regulatory hormones. Glucose levels will be checked using a home glucose meter every 15 minutes until the glucose level of $\leq$100 mg/dl is reached, after which glucose will be measured every 5 minutes and the test stopped once the glucose level reaches $\leq$60 mg/dl. Thereafter, the basal insulin rate will be restored, children will be treated with IV glucose to restore blood glucose and then eat breakfast prior to discharge. A significant proportion of patients will not report symptoms of hypoglycemia at this glucose level and progression to severe hypoglycemia is extremely unlikely with the careful monitoring that is planned. Given the careful safeguards in place, we would argue that the Subcutaneous Insulin Infusion Test poses no more than a minor increase over minimal risk for these children.

Further, there are a number of potential benefits that could accrue to participants. First, all participants will be able to use the Guardian RT for approximately 6 days prior to the CRC admission. This could reveal previously unrecognized glucose trends, particularly during postprandial periods and during sleep, thus enhancing diabetes management. Second, the study could reveal whether an individual patient mounts a counter-regulatory hormone response to mild hypoglycemia and/or slowly falling glucose levels. Such information could help to gauge the degree to which avoidance of hypoglycemia should be prioritized in that patient's diabetes management. Finally, the study will yield information about glycemic sensitivity to modest changes in basal insulin infusion rates that could inform subsequent self-management decision making by children and parents. Thus, the planned study offers the prospect of several meaningful direct benefits to participants as well as contributing valuable knowledge about counter-regulatory hormone action during mild hypoglycemia in two age groups of children with diabetes.

Our previously completed DirecNet protocol entitled "The Accuracy of Continuous Glucose Monitors in Children with Type 1 Diabetes" posed similar risks to study participants and affords the best perspective for evaluating the risks and benefits associated with the planned study. In that prior study, children between the ages of 1 and <18 years were admitted to the Clinical Research Center at a participating site for an approximate 26 hour hospitalization. Children wore two different kinds of continuous glucose monitors throughout those admissions (Cygnus GlucoWatch 2 Biographer and Medtronic Minimed CGMS) while serial blood samples were drawn for comparison of glucose

values obtained by those devices with central laboratory results. Blood samples were drawn through an indwelling heparin lock every 60 minutes during waking hours and every 30 minutes during sleep. Children also underwent an IV insulin challenge (≥7 years old only) and a meal challenge (all children) so that sensor accuracy could be evaluated during periods of rapidly falling and rapidly rising glucose levels, respectively. Carefully calculated limits ensured that the volume of blood samples drawn was safe based on each child's weight. There were no significant adverse events during this study. Skin reactions were associated with use of the GlucoWatch in a significant proportion of the children enrolled in that study, but that device will not be used in the planned study.

As in the previously completed study, the volume of blood samples to be collected will not exceed 5% of the subject's total blood volume.

In comparison to this previously completed study, the planned protocol includes a shorter CRC admission (18 vs. 26 hours).

5.5.1 Hypoglycemia

Subjects may develop hypoglycemia. Severe uncontrolled hypoglycemia may be associated with profuse diaphoresis, shock, tachycardia, and seizures. Hyperglycemia is usually acutely benign, but may be associated with thirst, glycosuria, ketoacidosis, and hyperosmolar coma. The risk of these complications is an inherent risk of having diabetes.

The purpose of this study is to examine the effect of age on hormonal responses to mild hypoglycemia (i.e. the study will be stopped when the plasma glucose level falls below 60 mg/dl). This plasma glucose level was selected because it is extremely unlikely to cause any major symptoms and because blood glucose levels considerably lower than 60 mg/dl are commonly experienced by children and adolescents with T1DM. In our recent DirecNet study(23), approximately 25% of children and adolescents had plasma glucose levels below 60 mg/dl during a typical night and this rose to approximately 50% of subjects when there was antecedent exercise in the late afternoon. Moreover, the frequency of mild as well as severe hypoglycemia is substantially higher in pre-school children than in children and adolescents. The insulin infusion doses have been designed to cause a gradual reduction in plasma glucose and the study will be performed in a controlled environment with a physician available. Plasma glucose levels will be measured every 5 minutes at the bedside when the plasma glucose level falls below 100 mg/dl. Once the glucose concentration reaches <60 mg/dl, hormone samples will be obtained and plasma glucose levels will be acutely raised by intravenous administration of glucose that will be available at the bedside. The study will also be terminated early if any of the children become very uncomfortable due to symptoms of hypoglycemia (e.g., sweaty, shaky, palpitations) at plasma glucose levels that are >60 mg/dl. Such close monitoring of plasma glucose levels ensures that there will be virtually no risk that the plasma glucose concentration will fall into a dangerous zone that might result in an alteration in level of consciousness or other sign or symptom of more severe hypoglycemia.

Based on the fact that (1) children with diabetes experience mild hypoglycemia frequently as a consequence of the disease and its management, (2) the study induces a gradual drop in blood glucose under careful medical monitoring, (3) a conservative glucose nadir of 60mg/dl will be carefully enforced with glucose determinations being made every 5-minutes, and (4) rapid reversal of hypoglycemia can be achieved it is the assessment of the investigators that this protocol falls under DHHS 46.405 which is a minor increase over minimal risk. In addition, it is the belief of the investigators that this study also presents prospect of direct benefit to the subjects and general benefit to others with diabetes as described in Section 6.1.

5.5.2 Guardian-RT

There is a low risk for developing a local skin infection at the site of the sensor needle placement. Itchiness, redness, bleeding, and bruising at the insertion site may occur. The FDA approval of the Guardian RT is for a 3-day wearing period for each sensor. Nonetheless, a longer wearing period should be safe, effective, and more acceptable to patients. With a longer wearing period, the risk of skin reactions may increase.

5.5.3 HypoMon

There are no known risks associated with use of the HypoMon.

5.5.4 Fingerstick Blood Glucose Measurements

Fingersticks may produce pain and/or ecchymosis at the site.

5.5.5 IV Risks

A hollow needle/plastic tube will be placed in the arm for taking blood samples or giving fluids during the CRC admission. This will be left in for approximately 18 hours. When the needle goes into a vein, it can cause pain. A special cream (Elamax or EMLA®) may be used to numb the area where the needle will be inserted. The most common risks related to putting the numbing cream on the skin are redness, blanching (temporary whiteness of the skin area), swelling, and itching. There will be the minor discomfort of having the needle/plastic tube taped to the arm. In about one in 10 cases a small amount of bleeding under the skin will produce a bruise. The risk of a blood clot forming in the vein is about one in 100, while the risk of infection or significant blood loss is one in 1000.

5.5.6 Blood Volume Requirements

At the time of CRC admission the maximum number of blood draws that can be performed based on a subject's weight will be determined so that the maximum blood volume in the blood draws will not exceed 5% of the subject's blood volume (calculated by multiplying the subject's weight in kilograms by 70 [70cc / kg blood volume] and then multiplying by .05). The maximum number of blood draws is then determined by dividing this maximum blood volume by the amount of blood in each blood draw at the center.

The table below shows the blood volumes for each procedure at the "reinfusion" and "discard" centers. For blood draws for evaluation of the accuracy of the Guardian RT a blood volume of 1.3 ml per blood draw at the "discard" centers and 0.3 ml per blood draw at the "reinfusion" centers is assumed. At the "discard" centers, the maximum number of blood draws per subject will be adjusted if the blood draw amount exceeds 1.3 ml.

Table 5.1 Blood Volume Requirements for Procedures According to Type of Blood Draw

		Type of Blood Draw Employed at the Clinical Center	
Procedure	**# of blood draws**	**"Reinfusion" (Volume to be drawn)**	**"Discard" (Volume to be drawn)**
A. Subcutaneous Insulin Infusion Test	6	44.5	60.5
		(0.3 ml per blood draw)	**(1.3 ml per blood draw)**
B. Half-Hourly measurements for 12 hrs	24	7.2	31.2
C. Quality control sample	1	0.3	1.3
D. Meal-induced hyperglycemia test	8	2.4	10.4

The tables below indicate the procedures to be done based on the age and/or weight of the subjects.

Table 5.2: Procedures to Be Done / Blood Volume Required According to Weight of Subject

A. "Reinfusion" Centers

	Procedure (*see description in Table 5.1 for each 'letter'*)				
Subject Weight	**A**	**B**	**C**	**D**	**Total Blood Volume**
12.8 to <14.9 kg	44.5	--	0.3	--	44.8
14.9 to 15.5 kg	44.5	7.2	0.3	--	52.0
≥15.6 kg	44.5	7.2	0.3	2.4	54.4

B. "Discard" Centers

	Procedure (*see description in Table 5.1 for each 'letter'*)				
Subject Weight	**A**	**B**	**C**	**D**	**Total Blood Volume**
17.4 to <26.3 kg	60.5	--	0.3	-	60.8
26.3 to 29.2 kg	60.5	31.2	0.3	-	92.0
≥29.3 kg	60.5	31.2	0.3	10.4	102.4

* based on adjusted schedule of every 30 min overnight (9PM – 6AM) and hourly at other times

The study may include other risks that are unknown at this time.

CHAPTER 6
MISCELLANEOUS CONSIDERATIONS

6.1 Benefits

It is possible that subjects will not directly benefit from being a part of this study. However, it is also possible that the blood sugar and hormone information will be useful for subjects' diabetes management. Specifically, the study will offer participants the benefit of determining for each individual child the glucose level that triggers symptoms and the different types of symptoms and, conversely, how their child appears and what symptoms they have when the blood glucose level falls below 60 mg/dl. This information is especially valuable in young children, who are not developmentally able to relate to their care givers how they feel. Parents could use this information to help them with the daily management of these children's diabetes at home and thus prevent hypoglycemia and its neurologic and possibly developmental sequelae. It is beneficial for individuals to learn their idiosyncratic responses to falling glucose levels and for their physicians to identify patients who fail to mount a counter regulatory hormone response to low or falling glucose levels. Glycemic targets, correction doses, etc. might be modified accordingly in an effort to prevent hypoglycemia in such patients.

Results of this study will also provide important new knowledge that will be generalizeable to all children with type 1 diabetes regarding defects in counter-regulatory hormone responses to hypoglycemia that make children in general and young children in particular vulnerable to hypoglycemia.

6.2 Subject/Parent Reimbursement

The study will provide the Guardian RT and related supplies and the study HGM and test strips.

The study will be paying $100 for the CRC admission and $25 for the study enrollment visit to cover travel and other visit-related expenses. Payment will not be made for missed visits. Payment will be made after the child completes the study.

At the end of the study, subjects will be able to keep the study HGM.

6.3 Subject Withdrawal

Participation in the study is voluntary, and a subject may withdraw at any time. The investigator may withdraw a subject who is not complying with the protocol.

6.4 Confidentiality

For security purposes, subjects will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the coordinating center, the Jaeb Center for Health Research in Tampa, FL. Information given to the coordinating center will include: diagnosis, general physical exam information (height/weight/blood pressure/etc.) insulin, questionnaire results, hemoglobin A_{1C} results, continuous glucose monitor results, blood work results, HGM blood glucose measurements, information pertaining to hypoglycemic excursions and the treatment given, as well as all other study related data gathered during study visits. During the CRC admission, the Guardian-RT, study HGM and subject's insulin pump (if downloadable) will be downloaded to a computer that is secured and password protected, the files will be sent directly to the coordinating center via email. All files will include only the subject's identifier; no names or personal information will be included. Laboratory specimens will be sent to the University of Minnesota which serves as the central lab for DirecNet. Specimens may also be sent to other laboratories.

During the study, subjects will be asked to wear the Guardian RT and use the study HGM which will be downloaded by the study personnel at the end of the CRC admission. The downloaded data will be emailed to the coordinating center. No names or personal information will be included. LifeScan Inc., the company providing the study HGMs and test strips for the study will be provided with the downloaded data from the study HGM. The data provided to LifeScan Inc. will include only the subject's identifier; no names or personal information will be included.

Chapter 7
Statistical Considerations

A detailed statistical analysis plan will be written and finalized prior to the completion of the study. The analysis plan synopsis in section 7.2 contains the framework of the anticipated final analysis plan, which will supersede section 7.2 when it is finalized.

7.1 Sample Size

Jones et al. (9) measured hormone levels in children with type 1 diabetes during induced hypoglycemia. The standard deviation (calculated by multiplying their reported standard error by the square root of the sample size and dividing by 5.46 to convert to pg/mL) for epinephrine was 306 pg/mL. With this standard deviation and a two-tailed test with type I error rate of 5%, a sample size of 50 subjects (25 in each age group) will give approximately 92% power to detect a mean difference of 300 pg/mL between the two age groups.

After N=30 subjects have completed the subcutaneous insulin infusion test an interim analysis will be performed to re-evaluate the sample size calculation above. The standard error for the age effect from the ANCOVA model for epinephrine described in section 7.2.1.1 will be calculated using the outcome data from the first 30 subjects. This will be used to update the calculation above to obtain the sample size necessary to detect a mean difference of 300 pg/mL with 90% power. Results will be presented to the DirecNet DSMB to determine whether the study should be stopped for either efficacy/futility or whether recruitment should continue to the newly calculated sample size (up to a maximum of 50 total subjects).

To avoid inflating the type I error rate with this interim analysis, the following stopping guidelines will be recommended: If N=30 subjects provides ≥80% power using the updated variance estimate or if N=50 would only increase this power by <5%, then no additional subjects will be recruited and statistical analysis will proceed without adjustment for alpha spending. Otherwise, Pocock's test will be used to determine whether to stop at N=30 or continue recruitment to the newly calculated sample size (up to a maximum of 50 total subjects). If conditional power based on the age effect observed so far is less than 30% then stopping for futility will be recommended.

Based on the results a pilot study of the FreeStyle Navigator™ (Abbott Diabetes Care) performed by DirecNet, a sample size of 30-50 subjects will result in approximately 1,800-3,000 sensor-reference pairs giving a 95% confidence interval for the median relative absolute difference no more than ± 1.0%. With this sample size the half width of the 95% confidence interval for the median difference will be approximately ± 5-6 mg/dL.

7.2 Analysis Plan

7.2.1 Counterregulatory Responses during Insulin-induced Hypoglycemia

7.2.1.1 Hormone Concentrations

Boxplots will be used to summarize the distribution of hormone levels separately for the two age groups by glucose level. An analysis of covariance model (ANCOVA) will be fit separately for each hormone with the change from baseline to peak during the subcutaneous insulin infusion test as the dependent variable. The model will include a term for age group (3-<7 vs. 12-<18) with baseline hormone concentration as the covariate.

A repeated measures regression model will also be fit treating each hormone measurement as a separate observation (instead of just using the peak value as above). Either an autoregressive or Toeplitz structure will be used to model the covariance pattern. Residual values will be checked for

approximate normality. If the distribution is highly skewed a transformation may be used. Suspected outliers will either be truncated or excluded.

Additional exploratory models will be fit to assess possible associations of counterregulatory responses with the following covariates:

- age as continuous
- HbA1c
- gender
- BMI percentile
- clinical center
- glycemic indices as measured by the Guardian RT during home use prior to the subcutaneous insulin infusion test (see section 7.2.2.1)

Continuous covariates will be modeled as linear in the dependent variable or divided into categories if significant non-linearity is detected. The number of hypoglycemic episodes per 24 hours measured by the Guardian RT will also be categorized as <2.0 vs. 2.0-<3.0 vs. ≥3.0.

7.2.1.2 Glucose Level Triggering a Counterregulatory Response

For each hormone, the glucose level at which a counterregulatory response was observed will be treated as a secondary outcome. A response will be defined as the first glucose level at which there was a sustained increase of the hormone concentration at least 3 standard deviations above baseline. If no such increase is observed, then the subject will be deemed not to have responded to hypoglycemia.

If the outcome values are not skewed a regression model with a normal distribution and interval censoring will be fit to the data using maximum likelihood. Age group and the baseline hormone value will be included as independent variables. If significant non-linearity is detected with the baseline hormone, it will be divided into categories and treated as a discrete variable. If the values show a skewed distribution then an alternate distribution such as a Weibull or lognormal will be fit by maximum likelihood. If no distribution can be found to give a good fit to the data, then a semi-parametric proportional hazards regression will be fit.

7.2.1.3 Hypoglycemic Symptoms

Analysis of physiologic symptom measures from the HypoMon (if used) and symptom scores reported by the parent will parallel the methods described in section 7.2.1.1. If residual values from the regression models have a highly skewed distribution, then transformations will be explored. If no suitable transformation can be found for the parent-reported symptom scores, then ordinal logistic regression will be used instead.

7.2.2 Continuous Glucose Monitor (CGM) Data

7.2.2.1 Glycemic Indices

Analyses based on the Guardian RT will be based on 5 minute readings.

Multiple sensor values <70 mg/dL within 30 minutes will be considered a single hypoglycemic episode. Similarly, multiple values >200 mg/dL within 30 minutes will be considered part of the same hyperglycemic episode.

The following glycemic indices to be calculated for each subject:

- Mean glucose.

- Percentage of readings between 70-180 mg/dL.
- Hypoglycemia:
  - Percentage of readings < 70, 60, 50 and 40 mg/dL.
  - Area over the curve (a composite measure of percentage and severity of sensor values ≤70mg/dL), defined as the area between the glucose curve and 70 mg/dL.
  - Number of hypoglycemic episodes (as defined above) per 24 hours.
- Hyperglycemia:
  - Percentage of readings > 180, 200, 250 and 300 mg/dL.
  - Area under the curve (a composite measure of percentage and severity of sensor values >200mg/dL), defined as the area between 200 mg/dL and the glucose curve.
  - Number of hyperglycemic episodes (as defined above) per 24 hours.

7.2.2.2 Accuracy

Laboratory glucose measurements will be used as the reference during the CRC Admission for determination of the accuracy of the Guardian-RT. The subject's glucose meter (excluding values used to calibrate the sensor) will be used as the reference during home use of the Guardian RT. Sensor readings will be paired to reference values within ± 2.5 minutes. For each pair the following difference measures will be calculated:

- Difference – Sensor minus reference value.
- Absolute Difference – Absolute value of the Difference.
- Relative Difference – Difference divided by the reference value.
- Relative Absolute Difference – Absolute value of the Relative Difference.
- ISO criteria – Sensor within ±15 mg/dL if reference ≤75 mg/dL, or sensor within ±20% if reference >75 mg/dL).

Percentiles will be given for the four difference measures and the percentage of pairs meeting the ISO criteria will be calculated. Results will be given separately for inpatient and outpatient data. Values will be given overall and stratified by reference glucose, age group, gender, HbA1c and BMI percentile. A Bland-Altman plot will be constructed. Confidence intervals and comparisons of subgroups will use the bootstrap to account for correlated data from the same subject.

For each subject the glycemic excursion during the subcutaneous insulin infusion test will be calculated separately for the reference and sensor values. This will be defined as the baseline minus the nadir glucose value. For the Guardian-RT, the nadir will be taken over the period from baseline to 60 minutes following completion of the subcutaneous insulin infusion test.

A scatterplot and Bland-Altman plot will be constructed to compare reference vs. CGM measured glucose drops during the subcutaneous insulin infusion test. Median values and quartiles will be given for each of the difference measures defined above. To investigate potential sensor lag, a scatterplot will be also constructed comparing the CGM vs. reference nadir times (defined as minutes from baseline). Summary statistics will be given for the difference of these two values.

A similar analysis will be done for the rise in glucose during the post-dinner blood draws described in section 4.2.

Sensitivity to detect hypoglycemia will be assessed by calculating the percentage of subjects with a sensor value <70, <60 and <50 mg/dL between baseline and 60 minutes following completion of the subcutaneous insulin infusion test. A similar sensitivity analysis for hyperglycemia will be done for those subjects with a reference glucose >200 mg/dL during the post-dinner blood draws.

During home use of the Guardian RT, analysis of hypoglycemia detection will be based on clinical events rather than individual points and will allow margins of error for both time (30 min) and glucose (10 mg/dL). Multiple glucose values below the hypoglycemic threshold (e.g., <70 mg/dL) within 30 minutes will be analyzed as a single event. Sensitivity will be defined as the percentage of reference events for which the sensor alarmed within 30 minutes of the first low reference value. The false positive rate will be defined as the percentage of sensor events for which all reference values within 30 minutes of the first low sensor reading were above the hypoglycemic threshold. Events where the sensor was always within ±10 mg/dL of the reference, but on the opposite side of the hypoglycemic threshold (e.g., sensor=68/reference=72) will not be counted as either a success or a failure and will be excluded from the calculation. Parallel analyses will be conducted for hyperglycemic episodes.

References

1. Cryer, P.E. and J.E. Gerich, Glucose counterregulation, hypoglycemia, and intensive insulin therapy in diabetes mellitus. N Eng J Med, 1985. 313(4): p. 232-41.

2. Cryer, P.E., S.N. Davis, and H. Shamoon, Hypoglycemia in diabetes. Diabetes Care, 2003. 26(6): p. 1902-12.

3. Becker, D.J. and C.M. Ryan, Hypoglycemia: a complication of diabetes therapy in children. Trends Endocrinol Metab, 2000. 11(5): p. 198-202.

4. Jones, T.W. and E.A. Davis, Hypoglycemia in children with type 1 diabetes: current issues and controversies. Pediatr Diabetes, 2003. 4(3): p. 143-150.

5. Ryan, C.M. and D.J. Becker, Hypoglycemia in children with type 1 diabetes mellitus. Risk factors, cognitive function, and management. Endocrinol Metab Clin North Am, 1999. 28(4): p. 883-900.

6. Davis, E.A. and T.W. Jones, Hypoglycemia in children with diabetes: incidence, counterregulation and cognitive dysfunction. J Pediatr Endocrinol Metab, 1998. 11(Suppl 1): p. 177-82.

7. Cryer, P.E., Diverse causes of hypoglycemia-associated autonomic failure in diabetes. N Eng J Med, 2004. 350(22): p. 2272-9.

8. Amiel, S.A., D.C. Simonson, R.S. Sherwin, A.A. Lauritano, and W.V. Tamborlane, Exaggerated epinephrine responses to hypoglycemia in normal and insulin-dependent diabetic children. J Pediatr, 1987. 110(6): p. 832-7.

9. Jones, T.W., S.D. Boulware, D.T. Kraemer, S. Caprio, R.S. Sherwin, and W.V. Tamborlane, Independent effects of youth and poor diabetes control on responses to hypoglycemia in children. Diabetes, 1991. 40: p. 358-63.

10. Dagogo-Jack, S.E., S. Craft, and P.E. Cryer, Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. J Clin Invest, 1993. 91(3): p. 819-28.

11. Davis, S.N., C. Shavers, F. Costa, and R. Mosqueda-Garcia, Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. J Clin Invest, 1996. 98: p. 680-91.

12. Davis, S.N., C. Shavers, B. Davis, and F. Costa, Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. J Clin Invest, 1997. 100: p. 429-38.

13. McGregor, V.P., J.S. Greiwe, S. Banarer, and P.E. Cryer, Limited impact of vigorous exercise on defenses against hypoglycemia: relevance to hypoglycemia-associated autonomic failure. Diabetes, 2002. 51(5): p. 1485-92.

14. Ryan, C.M., D. Dulay, C. Suprasongsin, and D.J. Becker, Detection of symptoms by adolescents and young adults with type 1 diabetes during experimental induction of mild hypoglycemia. Diabetes Care, 2002. 25(5): p. 852-8.

15. Jackson, L., F.L. Williams, A. Burchell, M.W. Coughtrie, and R. Hume, Plasma catecholamines and the counterregulatory responses to hypoglycemia in infants: a critical role for epinephrine and cortisol. J Clin Endocrinol Metab, 2004. 89(12): p. 6251-6.

16. Ross, L.A., R.E. Warren, C.J.H. Kelnar, and B.M. Frier, Pubertal stage and hypoglycaemia counterregulation in type 1 diabetes. Arch Dis Child, 2005. 90: p. 190-4.

17. Matyka, K.A., E.C. Crowne, P.J. Havel, I.A. Macdonald, D. Matthews, and D.B. Dunger, Counterregulation during spontaneous nocturnal hypoglycemia in prepurbertal children with type 1 diabetes. Diabetes Care, 1999. 22(7): p. 1144-50.

18. Bjorgaas, M., T. Vik, T. Sand, K. Birkeland, G. Sager, H. Vea, and R. Jorde, Counterregulatory hormone and symptom responses to hypoglycaemia in diabetic children. Diabet Med, 1997. 14(6): p. 433-41.

19. Jones, T.W., P. Porter, R.S. Sherwin, E.A. Davis, P. O'Leary, F. Fraser, G. Byrne, S. Stick, and W.V. Tamborlane, Decreased epinephrine responses to hypoglycemia during sleep. N Engl J Med, 1998. 338: p. 1657-62.

20. Hoffman, R.P., C. Singer-Granick, A.L. Drash, and D.J. Becker, Plasma catecholamine responses to hypoglycemia in children and andolescents with IDDM. Diabetes Care, 1991. 14: p. 81-8.

21. Brambilla, P., P.F. Bougneres, J.V. Santiago, J.L. Chaussain, A. Pouplard, and L. Castano, Glucose counterregulation in pre-school-age diabetic children with recurrent hypoglycemia during conventional treatment. Diabetes, 1987. 36: p. 300-4.

22. Monsod, T.P.C., W.V. Tamborlane, L. Coraluzzi, M. Bronson, T. Yong-Zhan, and J.A. Ahern, Epipen as an alternative to glucagon in the treatment of hypoglycemia in children with diabetes. Diabetes Care, 2001. 24(4): p. 701-4.

23. Diabetes Research in Children Network (DirecNet) Study Group, Impact of exercise on overnight glycemic control in children with type 1 diabetes. J Pediatr, 2005. 147: p. 528-34.

24. Boyne, M.S., D.M. Silver, J. Kaplan, and C.D. Saudek, Timing of changes in interstitial and venous blood glucose measured with a continuous subcutaneous glucose sensor. Diabetes, 2003. 52: p. 2790-4.

25. Steil, G.M., B. Bernaba, M. Saad, J.J. Mastrototaro, and K. Rebrin, Accurate determination of plasma glucose during hyper- and hypoglycemia with a subcutaneous glucose sensor (Abstract). Diabetes, 2000. 49(Supplement 1): p. A126.

26. Cox, D.J., A. Irvine, L. Gonder-Frederick, G. Nowacek, and J. Butterfield, Fear of hypoglycemia: quantification, validation, and utilization. Diabetes Care, 1987. 10(5): p. 617-621.

27. Clarke, W.L., L.A. Gonder-Frederick, A.L. Snyder, and D.J. Cox, Maternal fear of hypoglycemia in their children with insulin-dependent diabetes mellitus. J Pediatr Endocrinol Metab, 1998. 11 (Suppl 1): p. 189-194.

28. Green, L.B., T. Wysocki, and B.M. Reineck, Fear of hypoglycemia in children and adolescents with diabetes. J Pediatr Psychol, 1990. 15(5): p. 633-641.