The Effect of Exercise on the Development of Hypoglycemia in Children with Type 1 Diabetes

A study being conducted by the Diabetes Research in Children Network

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Table of Contents

CHAPTER 1
BACKGROUND INFORMATION AND STUDY SYNOPSIS

1.1 Background Information

The glycemic reduction benefits of exercise in patients with diabetes were recognized very early on, and since the days of Joslin and Allen, exercise has been recommended as one of the three cornerstones of diabetes management. Marble et al in 1936[1] showed that exercise decreases blood glucose levels. Subsequently, multiple studies in animals and humans have examined the metabolic and hormonal responses to exercise in diabetes mellitus.[2, 3] Studies have shown that both the short and long term benefits of exercise are desirable for patients with type 1 diabetes but the risks are also substantial.[4] Hypoglycemia during exercise in a child can be disruptive and may decrease the child's ability to perform during sports activities. Hypoglycemia following exercise, particularly nocturnal hypoglycemia during sleep the night following the day when exercise has occurred, is potentially dangerous and of great concern to parents and health care providers.

1.1.1 Exercise Studies in Children and Adolescents Related to Hypoglycemia Following Exercise

Studies examining the effect of exercise on blood glucose in children with T1DM are limited, particularly with regard to nocturnal hypoglycemia following exercise.

- Stratton et al[5] observed 130 exercise sessions in 8 adolescents with T1DM, over an eight-week period. Finger-stick blood sugars were checked before and after a 30-minute either structured aerobic or recreational exercise. The aerobic exercise session was on a treadmill or bike at 60-85% heart rate max and recreational was basketball, swimming, etc. without attention to heart rate. Blood glucose declined post exercise in both types of exercise but did not reach hypoglycemic levels. The decline was greater for higher pre-exercise blood glucoses. Late-onset post exercise hypoglycemia was not studied.

- Schiffrin et al[6] studied the effect of exercise on plasma glucose and free insulin in 13 adolescents with type 1 diabetes on intensive treatment (CSII and MSI). Plasma glucose and free insulin were determined postprandially, during 45 minutes of exercise and 45 minutes following exercise on four different occasions, 3 with varying doses of insulin administered before the preceding meal and one occasion with no insulin. The types of insulin used were NPH and regular (2-3 injections/day and regular insulin in the CSII). The free insulin levels did not change during exercise. Blood glucose declined according to the pre-meal insulin dose. The authors concluded that a 30-50% reduction of pre-meal insulin is appropriate for avoiding hypoglycemia during exercise performed 2 hours following a meal.

- Temple et al[7] evaluated the blood glucose response to prolonged exercise in 9 adolescent boys with T1DM. They found that 7 of the 9 subjects had decline of blood glucose during exercise. The higher the initial glucose, the more severe was the decline. This protocol called for exercise at the peak of insulin action as exercise was performed post-breakfast.

- Riddell et al[8] examined metabolic and substrate parameters in 20 adolescents with T1DM during exercise who were studied in two sessions 1-4 weeks apart. Exercise sessions were similar except for post-breakfast fluid consumption of either water or carbohydrate containing beverage. Blood was collected 10 minutes before and every 10 minutes up to 60 minutes after the start of exercise. The protocols consisted of either two 30-minute exercise periods separated by 5-minutes rest or six 10-minute exercise periods separated by 5-minutes rest each. Exercise was performed on a cycle ergometer and was at 55-65% maximal aerobic capacity for age group (heart rate 145-160). Results indicated less blood glucose decline during exercise in the glucose ingesting session.

- MacDonald[9] reported several cases of post-exercise late-onset hypoglycemia in young patients with T1DM. These episodes were mostly nocturnal hypoglycemia following exceptional exercise the preceding day. He also surveyed 300 young patients with T1DM, ages 4 to 24 years, over a 2-year period and reported that 48 experienced post-exercise, late-onset nocturnal hypoglycemia. Episodes of hypoglycemia were present regardless of blood glucose control, without evidence of obvious insulin peak at the time of hypoglycemia but always following exceptional but not standardized exercise during the preceding day.

1.1.2 Exercise Studies in Adults Related to Hypoglycemia Following Exercise

- King et al[10] reported a study assessing the effect of nocturnal hypoglycemia on well-being, cerebral function, and physical fatigue the next day in 10 adult subjects with T1DM. Subjects were admitted in a randomized order for one night of induced hypoglycemia and one during which blood glucose was maintained in a normal range. Exercise at 30 to 60% VO2 max during the following day assessed fatigue, and neuropsychological testing assessed cerebral function and well-being. There was no difference in these parameters following nocturnal hypoglycemia.

- Biankin et al[11] evaluated the reproducibility of plasma glucose during exercise in the fed and fasting state in 20 subjects with T1DM (treated with NPH and regular insulin) and found that the glucose pattern was more reproducible in the fasting state than in the fed state.

- Mauvais-Jarvis et al evaluated the effect of insulin reduction prior to intense exercise on plasma glucose during exercise in 12 subjects with T1DM.[12] A 60-minute high-intensity exercise at 70% VO2max was completed on two occasions, 90 minutes post-breakfast. On one occasion, the insulin dose was not adjusted and on the other occasion, the insulin dose was decreased by 50-90% depending on insulin regimen. When insulin was not reduced, 2/3 of subjects experienced hypoglycemia. Based on the results of the study, the authors recommended that insulin be decreased before athletic activities.

1.1.3 The Effect of Exercise on Hormones, Substrates and Counter-regulatory Hormones

When hypoglycemia occurs, a cascade of metabolic, neuroendocrine and autonomic nervous system (ANS) responses are activated in order to restore normoglycemia. An abundant body of data have accumulated regarding the development of impaired physiological responses to hypoglycemia in diabetic patients. In patients with type 1 diabetes, the glucagon responses to hypoglycemia, for e.g., are permanently lost shortly after the onset of the disease.[13] However, in the absence of autonomic neuropathy, the sympathetic nervous system responses are preserved and are able to initiate a counter-regulatory response to hypoglycemia. In 1975, Felig et al[14] summarized a number of studies done in adults during 45 minutes of exercise and described the novel observation of elevated glucagon during exercise. Nonetheless, after a hypoglycemic episode, counter-regulatory responses to subsequent hypoglycemia are often reduced, increasing the likelihood of impaired counter-regulatory responses to further hypoglycemic episodes. This phenomenon, known as "*hypoglycemia-associated autonomic failure"* has been observed in healthy volunteers and diabetic subjects.[15-20] Even in normal children, deep sleep causes severe impairments in counterregulatory hormone responses to hypoglycemia, which, in turn, may contribute to low glucose values on nights following periods of exercise.[21]

Recently, detailed physiological studies performed have shown that hypoglycemia and exercise may reciprocally impair each other's counter-regulatory responses. Antecedent hypoglycemia blunts the counter-regulatory responses to exercise, and vice versa, antecedent exercise blunts the counter-

regulatory responses to subsequent hypoglycemia. This has been shown in both healthy volunteers [22, 23] and type 1 diabetic subjects.[24] Although, as expected, epinephrine responses to exercise are decreased in diabetic patients with autonomic neuropathy,[25] even well-controlled diabetic patients can have blunted neuroendocrine responses to exercise.[26] The inability of type 1 diabetic patients to suppress insulin levels during exercise may be a factor responsible for exercise-induced hypoglycemia. During exercise, ANS activation and increase catecholamine secretion drive the increase in lipolysis and the release of gluconeogenic precursors (amino acids and lactate). Experiments conducted by Galassetti, et al[24] in young adults (mean age 28 years) with type 1 diabetes in good to fair control, showed that, after resting euglycemia, the patients had normal counter-regulatory responses to the exercise challenge. However, when exercise occurred after experimentally-induced hypoglycemia, there was a reduced ANS drive and catecholamine levels during the exercise, with blunted lactate and lipolytic responses. This was observed despite maintenance of euglycemia during exercise. Data thus far do not support a major role of impaired cortisol responses to hypoglycemia contributing to reduced ANS responses to exercise in diabetics,[27] but lesser levels of glucagon, catecholamines, GH, lactate and glycerol are clearly observed.

Hepatic glucose production (Glu Ra) and peripheral glucose uptake (Glu Rd) are also increased during exercise in order to meet the metabolic demands of skeletal muscle. It is believed that the glucagon to insulin ratio may be a key determinant of Glu Ra during moderate intensity exercise in diabetic subjects.[28] Experiments in type 1 diabetics have shown that if exercise is intense, Ra increases much more than the Rd, resulting in hyperglycemia during exercise.[29] When counter-regulatory hormone and substrate concentrations were measured outside the CRC setting, before and after either a triathlon, or prolonged cross-country skiing for example, and without control of the glycemic levels during exercise *per se*, investigators reported large interindividual variations in the increase in the levels of catecholamines and substrates during exercise in diabetics.[30] However, since the patients reduced their insulin doses substantially (30-40%) there was overall relative normoglycemia, suggesting that with appropriate adjustment of the insulin doses and diet, type 1 diabetics can participate in competitive endurance sports. Taken in aggregate, these data suggest that different strategies may be needed to compensate for the glycemic levels during and post exercise depending on the pre-exercise blood glucose concentrations and the intensity of the exercise.

All of the above reported studies have been conducted in adults and there is paucity of data on the effect of exercise on the counter-regulatory responses, both ANS and hormonal, in children and adolescents with type 1 diabetes.

1.2 Study Objectives

In this study protocol, each subject is hospitalized in the CRC for two days. During one of the two days (ordered through randomization), a structured exercise protocol is completed in the late afternoon. Most of the study objectives involve a within-subject comparison of data collected on the exercise day versus the sedentary day.

1.2.1 The Relationship of Exercise and Hypoglycemia

The primary objective of the study is to determine the frequency/intensity of nocturnal hypoglycemia following exercise.

- The primary study question to be addressed is: Does exercise in the late afternoon increase the frequency/intensity of nocturnal hypoglycemia?

Additional objectives relate to the effects of exercise on the glucose level during and following exercise. Questions to be addressed include the following:

- What is the frequency of hypoglycemia during and immediately following exercise?
- What is the time course of a decrease in blood glucose during and immediately following exercise?
- What is the frequency of blood glucose elevation during exercise?
- Does exercise in the late afternoon increase the frequency of blood glucose values in the target range and reduce deviation from ideal prior to dinner, following dinner, and overnight?
- Does exercise reduce the frequency/intensity of hyperglycemic episodes?
- What factors are predictive of hypoglycemia during exercise and delayed nocturnal hypoglycemia following late afternoon exercise?

1.2.2 The Relationship of Exercise and Counter-regulatory Hormones and Plasma Substrate Concentrations

Objectives include the following:

- Determination of the changes in glucagon concentrations during exercise and overnight following exercise
- Determination of the changes in epinephrine concentrations during exercise and overnight following exercise

1.2.3 The Accuracy of a Continuous Glucose Sensor During Exercise

The accuracy of a continuous glucose sensor during exercise will be compared with its accuracy during sedentary periods.

1.2.4 The Accuracy of a Home Glucose Meter

The accuracy of a home glucose meter may be examined. There will be no additional blood requirements to perform this testing.

1.3 Synopsis of Study Design

Study Population: 75 subjects between 10.0 and <18.0 years old with HbA1c <10.0%, with each of the five clinical centers enrolling 15 subjects.

<u>Study Procedures</u>

1. Each subject will have two inpatient stays 1 to 4 weeks apart, each lasting about 24 hours: one sedentary and one with a 75-minute exercise session in the late afternoon. (The order of the exercise and sedentary days will be determined at random.)
2. Prior to each CRC admission, each subject will keep a one-week detailed diary of insulin use and hypoglycemia.
3. On each of the two admissions, the insulin regimens and diet will be as similar as possible.
4. On each of the two admissions, the following will occur:
   - Subjects will complete a questionnaire regarding what exercise he or she has had in the previous 3 days.
   - A continuous glucose sensor will be inserted and calibrated one hour later.

- An intravenous catheter will be inserted for the reference and Ultra glucose measurements and collection of counter-regulatory hormone and plasma substrate samples.
- BG measurements will be made with an Ultra meter every half hour beginning at 10:00 p.m. through 6:00 a.m.
- Blood samples for glucagon, epinephrine, and glucose will be collected hourly from 10:00 p.m. to 6:00 a.m.
- To assess accuracy of an alternative brand of home glucose meter, BG measurements may be made with the alternative home glucose meter each time a measurement is performed using an Ultra meter.

5. On the exercise day only, the subject will run on the treadmill in the morning for 5 to 15 minutes to determine the settings needed to achieve a heart rate of 140.
6. Exercise will begin at approximately 4:00 p.m. and will consist of 15 minutes on a treadmill at a heart rate of approximately 140 followed by a 5-minute rest period. This cycle will be repeated 3 more times for a total of four 15-minute exercise periods with 5-minute rest periods in between (75 minutes total). A heart rate monitor will be worn throughout the time of exercise to ascertain the effort put forth.
   - BG measurements will be made using the Ultra meter (1) prior to starting the exercise, (2) during each of the 3 rest periods, (3) immediately following the exercise session, and (4) at 15-minute intervals for one hour following the completion of the exercise. Blood samples will be collected for the central lab at the times of sampling for glucagon and epinephrine.

CHAPTER 2
SUBJECT ELIGIBILITY AND ENROLLMENT

2.1 Study Population

Enrollment of approximately 75 subjects is planned, with each of the five clinical centers enrolling 15 subjects. As noted in section 4.2.1, there is uncertainty with regard to the variance of the hypoglycemia index that is serving as the primary outcome, the correlation of the overnight glucose data for the exercise and sedentary days, and the frequency of overnight hypoglycemia on the sedentary day. Therefore, an interim analysis is planned after the completion of the enrollment of approximately 35 subjects to evaluate whether the parameters used in the sample size estimation appear accurate or if the sample size should be adjusted.

Subjects will include both males and females and an enrollment goal will be to achieve an equal sex distribution.

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

Subjects who do not complete the protocol for both the exercise and sedentary days will be replaced in the enrollment quota.

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 for at least 18 months
   The diagnosis of type 1 diabetes is based on the investigator's judgment; C peptide level and antibody determinations are not needed.
2) HbA1c $\leq$10.0%
   The DCA2000 will be used to assess eligibility.
3) Age 10.0 to <18.0 years
4) Weight $\geq$36.0 kg
5) BMI $\geq 5^{th}$ and $\leq 95^{th}$ percentiles for age and gender
6) Stable insulin regimen for at least 1 month and not anticipating a change prior to the subject's completion of the study
   - *Stable is defined as no change in the overall insulin program, i.e., no change from SC injections to pump or Lantus therapy, or Lantus therapy to pump.*
7) Insulin regimen involves either use of an insulin pump or Lantus (with short-acting insulin)
8) NPH or Lente, if part of the insulin regimen, is given only in the morning before breakfast
9) Normal hematocrit (within normal limits of local laboratory)
10) Normal thyroid function (measured within the previous year)
11) Parent/guardian and subject understand the study protocol and agree to comply with it
12) Informed Consent Form signed by the parent/guardian and Child Assent Form signed (*unless IRB requirements differ)*

2.2.2 Exclusion

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

1) Insulin regimen includes Ultralente/Lente or NPH at times other than the morning before breakfast
2) A recent injury to body or limb, Addison's disease, muscular disorder, use of any medication or other significant medical disorder if that injury, medication or disease in the judgment of the investigator will affect the completion of the exercise protocol
3) Asthma which has been medically treated within the last year
4) Current use of glucocorticoid medication (by any route of administration)
5) Current use of a beta blocker medication
6) Use of pseudoephedrine 48 hours prior to CRC admission (if used in the 48 hours prior to the scheduled second admission, the admission will be deferred)
7) Severe hypoglycemia resulting in seizure or loss of consciousness in the 2 weeks prior to CRC admission (if a severe episode occurs within 2 weeks prior to the scheduled second admission, the admission will be deferred)
8) Active infection (if at the time of the planned second admission an infection is present, the admission will be deferred)
9) Anticipating a significant change in exercise regimen between admissions (i.e. starting or stopping an organized sport)

2.3 Subject 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 Informed Consent

For eligible subjects, the study will be discussed with the subject and parent/legal guardian. The parent will be provided with the Informed Consent Form to read and will be given the opportunity to ask questions. Subjects 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 participation, the Informed Consent Form and Child Assent Form will be signed and the first inpatient hospital stay will be scheduled. 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.

2.3.1.1 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.

2.3.1.2 Special Consent Issues

The study population for this study includes 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. 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.

2.3.2 Historical Information

A history will be elicited from the subject and parent and extracted from available medical records. Data to be collected will include: age, gender, race, diabetes history, history of diabetes in other family members, current insulin management, other chronic conditions, use of other medications, and medication allergies.

2.3.3 Physical Exam

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). The physical exam will include Tanner staging of breast development and pubic hair in females and genital development and pubic hair in males.

CHAPTER 3
INPATIENT STUDY PROCEDURES AND MANAGEMENT

3.1 Overview

The study will consist of the following:

1) Two inpatient stays each lasting about 24 hours: one sedentary and one with a 75-minute exercise session in the late afternoon.
   - The order of the exercise and sedentary days will be determined at random.
2) Assessment of changes in glucose concentrations during and following exercise.
3) Assessment of changes in epinephrine and glucagon concentrations during and following exercise:

The first CRC admission should occur within 1 to 4 weeks of the subject's enrollment into the study. The second CRC admission should occur between 1 and 4 weeks of the first admission.

- If the subject experiences a severe hypoglycemia episode after the completion of the first hospital day but prior to the second hospital day, the second hospitalization will be deferred until at least 2 weeks after the episode.
- If the subject is ill at the time of the planned second admission, the admission will be deferred.

On each of the two admissions, the insulin regimens and diet will be as similar as possible. Either prior to the first admission or prior to lunch on the day of the first admission, the subject's meals will be planned and algorithms used at home recorded for insulin correction doses at meals and before bed. In addition, the algorithm to be used for treating hypo or hyperglycemia present at 2 p.m. to achieve a target glucose level of 150 mg/dL (see section 3.2.5) will be recorded.

Insulin management on both the exercise day and sedentary day will follow the same routine that the subject is following at home. Correction doses at meals will be based on a sedentary day at home in cases where the subject alters the correction dose after exercise.

3.2 Study Protocol

All procedures in the following sections, with the exception of section 3.2.6 and its subsections, refer to both CRC admissions. A flow chart at the end of this chapter provides a timeline example of the protocol.

3.2.1 Study Procedures Prior to CRC Admission

Prior to or at the start of the first CRC admission, a form will be completed which will record the subject's usual carbohydrate to insulin ratios and insulin/kg. The subject will also select meals and snacks to be consumed during both admissions (the same content and amounts will be consumed at both visits).

Prior to each CRC admission, each subject will keep a detailed diary of insulin use and hypoglycemia for the week prior to admission.

For the morning of each CRC admission, the subject will be instructed to check the FBG level by meter, take his/her usual pre-breakfast insulin dose, and eat breakfast. For subjects on injection therapy, the same doses of intermediate and long acting insulin should be given on both study days. The dose of rapid-acting insulin can vary. The subject will be instructed to place the injection in a site other than the legs on the study days.

3.2.2 CRC Admission

On both the sedentary and exercise days, the subject will be admitted to the CRC prior to lunch. The timing of the admission will enable the subject to have lunch in the CRC at approximately 12 noon.

Immediately following the CRC admission, blood or urine ketone levels will be assessed on the subject. The hospitalization will be deferred if the urine ketone levels are >small or blood ketones are >1.0 mmol/L.

3.2.3 Initial CRC Procedures

Prior to lunch, the following will be done on both the exercise and sedentary days unless otherwise noted:

1) A questionnaire regarding the exercise the subject has performed during the previous 3 days will be completed.

2) On the exercise day only, the subject will run on the treadmill for 5 to 15 minutes to determine the settings needed to achieve a heart rate of 140.

3) For subjects using a pump, study staff will supervise the filling of a new reservoir and infusion set and the insertion of a new subcutaneous catheter in a site other than the leg.

    - If at anytime during the admission the BG is >300 mg/dL and moderate or large urine ketones are present or the blood ketone level is >1.0 mmol/L, the pump site can be changed at investigator discretion.

4) A continuous glucose sensor will be inserted and calibrated.

5) An intravenous catheter for the reference glucose measurements and collection of counter-regulatory hormone and plasma substrate samples will be inserted.
    - The intravenous catheter will be inserted in an arm vein. The area where the catheter will be inserted may be numbed with cream prior to catheter insertion.

3.2.4 Procedures Related to Lunch

The blood glucose level will be checked using the Ultra meter about 30 minutes prior to lunch, which will be served at about 12 noon.

The pre-lunch bolus dose of rapid-acting insulin analog will be calculated based on the carbohydrate to insulin ratio and correction factor that the subject uses at home.

If BG level is:

- <60 mg/dl, give 10-15 grams of glucose as glucose tablets and recheck BG level in 15 minutes. Repeat as needed to raise BG value to >60 mg/dl.
- 60-150, give bolus dose 0-5 minutes prior to lunch
- 150-300, give bolus dose 15 minutes prior to lunch
- >300, check blood or urine ketones. If ketones are negative, give bolus dose 30 minutes prior to lunch. If ketones positive, recalculate correction dose and administer new pre-lunch bolus a least 30 minutes before lunch. Recheck BG level after 30 and, if needed, after 60 minutes to ensure that BG levels are decreasing. Check blood or urine ketones every 60 minutes until negative.

3.2.5 Post Lunch Procedures

At about 2:00 p.m., the BG will be checked with the Ultra meter.

- If the post-prandial glucose level indicates that the 4:00 p.m. BG level may be greater than 200 mg/dl, a correction dose of rapid-acting insulin analog can be given at the discretion of the investigator.
- If the post-prandial glucose level indicates that the 4:00 p.m. glucose may be <80 mg/dl, a snack consisting of 15-30 grams of carbohydrate can be given at the discretion of the investigator.

At about 3:00 p.m. and again at about 4:00 p.m., the BG will be checked with the Ultra meter.

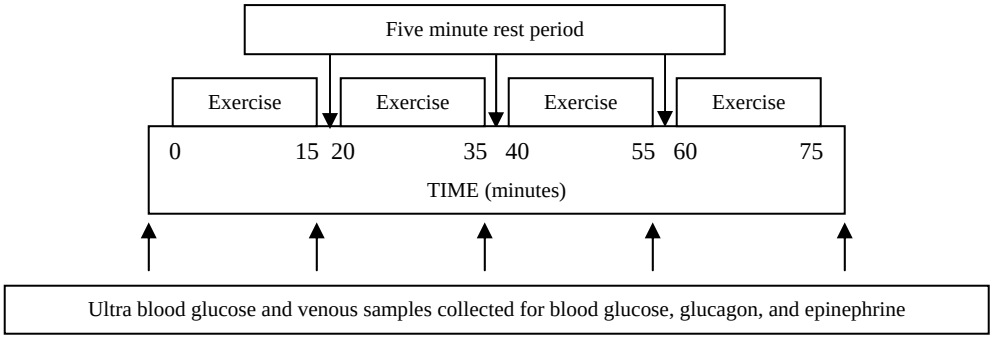
- If the glucose level is <80 mg/dL, the subject will be given 15g of carbohydrates and the BG will be rechecked every 15 minutes until it is $\geq$ 80 mg/dl
- If the glucose level is >300 mg/dL, the blood or urine will be checked for ketones. If there is a moderate or large urine ketone level or >1.0 mmol/L on blood ketone test, the study day will be discontinued and the subject will be discharged from the CRC and the admission rescheduled.

3.2.6 Exercise Procedures

On the exercise day, the exercise session will begin at about 4 p.m.

For subjects using an insulin infusion pump, the basal rate will be continued during the exercise.

Exercise will consist of 15 minutes on a treadmill at a heart rate of approximately 140 followed by a 5-minute rest period. This cycle will be repeated 3 more times for a total of four 15-minute exercise periods with 5-minute rest periods in between (75 minutes total). Subjects will be encouraged to complete the exercise but will not be coerced to complete any remaining cycles if they are unable. If the 4 cycles are not completed in 2 hours, the exercise will be stopped. A heart rate monitor will be worn throughout the time of exercise to ascertain the effort exerted.



If during exercise the BG drops to <60 mg/dL the subject will be given 15g of carbohydrate and after 5-15 minutes, the BG will be rechecked. Exercise will not resume until the BG is >70 mg/dL.

3.2.6.1 Glucose Measurements During Exercise

BG measurements will be made using the Ultra meter (see section 3.3.1) (1) prior to starting the exercise, (2) during each of the 3 rest periods, (3) immediately following the exercise session, and (4) at 15 minute intervals for one hour following the completion of the exercise. Blood samples for glucose will be collected for the central lab at the times of sampling for glucagon and epinephrine.

If the BG is <60 mg/dL, the subject will be given 15g of carbohydrates and rechecked prior to resuming exercise. No treatment will be given if the Ultra value is ≥60.

3.2.6.2 Blood Samples for Counter-Regulatory Hormones During Exercise

Blood samples will be collected for epinephrine and glucagon prior to starting the exercise, during each of the 3 rest periods, immediately following the exercise session, and 30 minutes after completion of the exercise session. The pre-exercise samples will be collected in duplicate.

3.2.7 Procedures Related to Dinner

The blood glucose level will be checked using the Ultra meter about 30 minutes prior to dinner, which will be served at about 6:15 p.m.

The pre-dinner bolus dose of rapid-acting insulin analog will be calculated based on the carbohydrate to insulin ratio and correction factor that the subject uses at home.

If BG level is:

- <60 mg/dl, give 10-15 grams of glucose as glucose tablets and recheck BG level in 15 minutes. Repeat as needed to raise BG value to >60 mg/dl.
- 60-150, give bolus dose 0-5 minutes prior to dinner
- 150-300, give bolus dose 15 minutes prior to dinner
- >300, check urinary or blood ketones. If ketones are negative, give bolus dose 30 min prior to dinner. If ketones positive, recalculate correction dose and administer new pre-dinner bolus a least 30 minutes before dinner. Recheck BG level after 30 minutes and, if needed, 60 minutes to ensure that BG levels are decreasing. Check blood or urine ketones every 60 minutes until negative.

After dinner, the blood glucose will be checked with the Ultra meter at 7:00 p.m., 8:00 p.m., and 9:00 p.m.

3.2.8 Procedures Related to Bedtime

A bedtime snack will be given at approximately 9:30 p.m.

- Meal planning prior to the first admission will include the content of the bedtime snack on both study days. This will be based on what the subject usually has for a bedtime snack on a non-exercising day (as well as no snack if that is the subject's usual routine). If the subject uses an algorithm at home for determining the size of the snack (or no snack), this will be followed.

- Insulin doses for the bedtime snack will also be based on what the subject usually does on a non-exercising day. This can be no extra insulin, cover the carbohydrate content but no correction dose (or visa versa), or cover both carbohydrates and correction.

- The same snack algorithm and the same insulin dose algorithm will be used on both days.

3.2.9 Overnight Procedures

Subjects will be asked to go to sleep at approximately 10:00 P.M. and will be awakened at approximately 7:00 A.M.

BG measurements will be made with the Ultra meter every half hour beginning at 10:00 p.m. through 6:00 a.m.

Treatment of hypoglycemia is described in section 3.3.3.

Blood samples for glucagon, epinephrine, and glucose will be collected hourly from 10:00 p.m. to 6:00 a.m.

3.2.10 Procedures Prior to Hospital Discharge

The CRC admission will be completed by approximately 8:30 A.M. Subjects will be provided breakfast and instructed on subsequent blood glucose monitoring and insulin use at home.

Prior to leaving the CRC, the IV and continuous glucose sensor will be removed.

3.3 Miscellaneous Protocol Issues

3.3.1 Glucose Measurements with the Ultra Meter

The Ultra meter will be used for the glucose measurements using venous blood from the intravenous catheter or from a fingerstick.

Three Ultra meters will be used for simultaneous measurement of the blood glucose level upon admission to the CRC. The meter with the median result of the 3 glucose values will be used to perform the measurements for the study.

If at anytime after the start of the exercise period on the exercise day or after the bedtime snack on the sedentary day the Ultra glucose measurement is <60 mg/dl at a time that a blood draw for the central lab is not scheduled, a specimen will be drawn for a central lab glucose measurement for confirmation. Blood for epinephrine and glucagon will also be collected at these times.

3.3.2 Continuous Glucose Sensor

A continuous glucose sensor will be used (1) to compare accuracy during exercise and non-exercise time periods and (2) to serve as a secondary outcome measure of hypoglycemia.

The guidelines provided by the manufacturer will be followed regarding sensor insertion, calibration values, and assurance of proper sensor function.

3.3.3 Treatment of Hypoglycemia

If a subject experiences symptoms of hypoglycemia and testing with the Ultra meter indicates a value less than 60 mg/dl, the subject will be given 15g of carbohydrates and a recheck of the blood glucose will be performed in 15 minutes. If the blood glucose value is still <60 mg/dl after 15 minutes, another 15g of carbohydrates will be administered. Checks will be done every 15 minutes and 15g of carbohydrates administered until the blood glucose value is >70 mg/dl. No treatment will be given if the value is $\geq$60 mg/dl.

3.3.4 Treatment of Hyperglycemia

Management when the BG is >300 mg/dl prior to 4 p.m. is described in section 3.2.5.

A BG >300 mg/dl detected pre-meal or at bedtime will be addressed using the dose algorithm the subject normally would use at home.

For a BG >300 mg/dl detected overnight, either blood or urine ketones will be checked. If moderate or large urine ketones are present or blood ketones are >0.5 mmol/L, insulin will be given; otherwise no insulin will be given.

3.3.5 Diet

Prior to the first admission, a diet will be planned for the subject for lunch, dinner, and the bedtime snack based on the subject's typical food intake at home. This diet plan will be followed on both the exercise and sedentary days.

All food intake at meals and at other times (e.g., treatment of hypoglycemia) will be recorded.

3.3.6 Daily Activities

Afternoon activities during both admissions will be calm, i.e. completing questionnaires, watching TV, or playing video games. Mobility will be limited to the CRC area.

3.3.7 Blood Samples for Additional Analyses

A portion of the blood sample taken for the glucagon, epinephrine, and glucose measurements by the central lab will be frozen and stored for possible later analyses, such as for insulin and hormones related to glucose regulation such as norepinephrine, cortisol, glycerol, free fatty acids, and others.

3.4 Risks

3.4.1 Exercise Risks

The exercise test involves exercising for a short time while pulse and blood sugars are monitored. It is routinely used to diagnose heart and lung problems. Four in 10,000 people get abnormal heartbeats or chest pain while doing this test. One in 100,000 people die. These are usually older people who have a history of heart conditions.

3.4.2 Fingerstick Risks

About 1 drop of blood will be removed by finger stick for measuring blood sugars. This is a standard method used to obtain blood for routine hospital laboratory tests. Pain is common at the time of lancing. In about 1 in 10 cases a small amount of bleeding under the skin will produce a bruise. A small scar may persist for several weeks. The risk of local infection is less than 1 in 1000. We recommend children with diabetes check their blood sugar at least 4 times daily. This should not be a significant contributor to risks in this study as finger pokes are part of the usual care for people with diabetes.

3.4.3 IV Risks

A hollow needle/plastic tube will be placed in the arm for taking blood samples or giving fluids. This will be left in for 24 hours. When the needle goes into a vein, it can cause pain. A special cream (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.

3.4.4 Subcutaneous Catheter Risks (Continuous Glucose Sensor)

Subjects using the continuous glucose sensor will be at low risk for developing a local skin infection at the site of the sensor needle placement. If a catheter is left under the skin for more than 24 hours it is possible get an infection where it goes into the skin, with swelling, redness and pain. There may be bleeding where the catheter is put in and bleeding under the skin causing a bruise (1 in 10 risk).

3.4.5 Risk of Hypoglycemia

As with any person having insulin-dependent diabetes, there is always a risk of having a low blood sugar (hypoglycemia) and of ketoacidosis. In this study, hypoglycemia may occur during or following the time the exercise portion of the study. Symptoms of hypoglycemia can include sweating, jitteriness, and not feeling well. Just as at home, there is the possibility of fainting or seizures (convulsions) and that for a few days you may not be as aware of symptoms of low blood sugar. Since we will be closely monitoring subjects during this study, a serious low blood sugar is not expected to occur. Even if severe low blood sugar does occur, it almost always goes away quickly with treatment to raise the blood sugar.

3.4.6 Blood Volume Requirements

At the time of 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.

A minimum blood volume of 3.53 ml per blood draw will be required at all centers. This will provide an extra 2.0 ml per draw for later analyses for the reinfusion centers and an extra 1.0 ml per blood draw for discard centers. For reinfusion centers, the blood sampling will remove approximately 65.07 ml of blood on the exercise day and 42.36 ml of blood on the sedentary day. For the discard centers, the blood sampling will remove approximately 73.07 ml of blood on the exercise day and 50.36 ml of blood on the sedentary day. This blood volume is acceptable for subjects weighing $\geq$36 kg.

At the discard centers, if a subject weighs at least 40.0 kg, an additional 0.5 ml per blood draw (above the 3.53 ml being collected for all subjects) will be collected for additional analyses, which increases the blood volume to 82.07 ml on the exercise day and 56.36 on the sedentary day (total of 138.43 ml). Subjects weighing at least 44.0 kg will have an additional 1.0 ml per blood draw (above the 3.53 ml being collected for all subjects) to provide 2.0 ml for additional analyses for a blood volume of 91.07 ml on the exercise day and 62.36 ml on the sedentary day (total of 153.43 ml).

		Type of Blood Draw Employed at the Clinical Center			
	Maximum # of blood draws	**"Reinfusion" (3.53 ml per blood draw)**	**"Discard" (3.53 ml per blood draw)**	**"Discard" (4.03 ml per blood draw)**	**"Discard" (4.53 ml per blood draw)**
Procedure		*blood volume (ml)*			
A. Hourly overnight samples for 9 hrs (2 days)	**18**	**63.54**	**63.54**	**72.54**	**81.54**
B. Blood draws during exercise (1 day)*	**7**	**22.71**	**22.71**	**25.71**	**28.71**
C. Blood draws for hypoglycemia (2 days)	**6**	**21.18**	**21.18**	**24.18**	**27.18**
		Blood Draws for Ultra Tests when Lab Sample is Not Being Collected (1.0 ml per blood draw)			
D. Half-hour overnight samples (10:30-5:30)	**16**	**N/A**	**16.0**	**16.0**	**16.0**
TOTAL		**107.43**	**123.43**	**138.43**	**153.43**

*A duplicate blood sample will be collected prior to the start of exercise. This duplicate sample will only require 1.53 ml at both reinfusion and discard centers. Therefore there will be 6 blood draws at a minimum of 3.53 ml and 1 draw at 1.53 ml.

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

3.5 Adverse Events

Adverse event reporting will be limited to (1) events that meet criteria for a serious adverse event (SAE), (2) events that are considered to have a possible (or greater) relationship to any study procedure, (3) hyperglycemia resulting in diabetic ketoacidosis or hyperosmolar nonketotic coma, and (4) hypoglycemia resulting in seizures or loss of consciousness. Adverse events that occur during the study and up to 1 week after completion of the second CRC admission will be reported.

An adverse event is considered a *Serious Adverse Event* (SAE) when it meets one or more of the following criteria: (1) death, (2) life-threatening, (3) required or prolonged hospitalization, (4) permanent disability, or (5) required intervention to prevent permanent impairment/damage.

The relationship of any adverse event to any aspect of study participation will be assessed and graded by a study investigator on a four-point scale: (1) not related, (2) possible, (3) probable, and (4) definite. 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.

3.6 Reporting Requirements for Serious and/or Unexpected Adverse Events

Any serious or unexpected adverse event occurring during the study and up to 1 week after completion of the second CRC admission will be reported to the Coordinating Center within one working day of occurrence. A written report on such an event will be sent to the Coordinating Center within five days of occurrence, stating a description of the reaction, any required intervention and the outcome. 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. Contact information for the Coordinating Center is located in the front of the protocol as well as in the Study Directory.

3.7 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) provides study oversight for all DirecNet protocols. The DSMB includes three physicians with expertise in type 1 diabetes in children, a statistician, and a psychologist. The DSMB meets at least twice each year either at a meeting or via conference call. The Board will review all serious adverse events on an expedited basis and will review all other adverse events as part of interval reports. The DSMB also will have a role in reviewing data as part of an interim analysis for sample size reestimation (see section 4.3).

3.8 Benefits

It is expected that the information gained from this study of exercise will have an important role in the management of diabetes in children. Therefore, the results of this study are likely to be beneficial for children with diabetes. In addition, it is possible that the blood glucose and counter-regulatory hormone response information will be useful for the subject's diabetes management by identifying how much these vary during and after exercise.

3.9 Subject Compensation

Subjects will receive $100 for each CRC stay for a total of $200 for completion of the two CRC admissions. Subjects who complete only one CRC stay will receive $100.

3.10 Data 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. At the end of each admission, the study devices 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.

825 Sample CRC Schedule*

Time	**Procedure**
10:00 AM	1. Subject admitted to CRC ➢ Check for blood or urine ketones ➢ Complete exercise questionnaire for previous 3 days ➢ Run on the treadmill for 5 to 15 minutes to determine the settings needed to achieve a heart rate of 140 ➢ For subjects using a pump, study staff will supervise the filling of a new reservoir and infusion set and the insertion of a new subcutaneous catheter in a site other than the leg. ➢ A continuous glucose sensor will be inserted and calibrated. ➢ An intravenous catheter will be inserted in an arm vein for the reference glucose measurements, Ultra glucose measurements, and collection of counter-regulatory hormone and plasma substrate samples. ➢ Check blood glucose on 3 Ultra meters (the Ultra with the median value will be used for the study Ultra testing).
11:30 AM	2. Blood glucose level checked using the Ultra meter about 30 minutes prior to lunch and pre-lunch insulin given as needed
12:00 PM	3. Lunch served
2:00 PM	4. BG checked with the Ultra meter and correction dose of insulin or carbohydrates given as needed
3:00 PM	5. BG checked with the Ultra meter and carbohydrates given or ketones checked as needed
4:00 PM	6. BG checked with the Ultra meter and carbohydrates given or ketones checked if needed
	7. BG permitting, exercise begins at 4 p.m. ➢ A heart rate monitor is put on the subject ➢ Pre-exercise duplicate blood samples are drawn for epinephrine, glucagon, and blood glucose ➢ The subject exercises for 15 minutes on a treadmill at a heart rate of approximately 140 followed by a 5-minute rest period during which a blood sample is drawn for epinephrine, glucagon, and blood glucose ➢ This cycle is repeated 3 more times for a total of four 15-minute exercise periods with 5-minute rest periods in between (75 minutes total). Blood samples are drawn for epinephrine, glucagon, and blood glucose during each 5 minute rest period and at the end of the 75 minutes.
5:15 PM	8. Exercise ends; BG checked with the Ultra meter
5:30 PM	9. BG checked with the Ultra meter
5:45 PM	10. Thirty minutes after the exercise has ended, an additional blood sample is drawn for epinephrine, glucagon, and blood glucose
5:45 PM	11. Blood glucose level checked using the Ultra meter about 30 minutes prior to dinner and pre-dinner insulin given as needed
6:15 PM	12. Dinner served; BG checked with the Ultra meter for last post-exercise check
7:00 PM	13. BG checked with the Ultra meter
8:00 PM	14. BG checked with the Ultra meter
9:00 PM	15. BG checked with the Ultra meter
9:30 PM	16. A bedtime snack will be given and insulin dose is given as needed
10:00 PM	17. Subjects will be asked to go to sleep

10:00 PM – 6:00 AM	18. BG measurements will be made with the Ultra meter every half hour
10:00 PM – 6:00 AM	19. Blood samples for glucagon, epinephrine, and reference glucose will be collected hourly
7:00 AM	20. Subjects will be awakened
7:30 AM	21. Blood glucose level checked using the Ultra meter about 30 minutes prior to breakfast
8:00 AM	22. Subjects provided breakfast and instructed on subsequent blood glucose monitoring and insulin use at home
8:30 AM	23. The IV and continuous glucose sensor are removed and the subject is discharged.

*For flow chart purposes, the schedule listed is for the exercise visit – the order of the visits will be randomized for each subject; the sedentary visit is identical to the exercise visit with the exception of running on the treadmill just after admission for determination of required exercise settings and running on the treadmill between 4:00 and 5:15 PM, which are only done on the exercise day.

CHAPTER 4
STATISTICAL CONSIDERATIONS

4.1 Statistical Analysis

The analysis plan is summarized below. It will be detailed in a separate document.

4.1.1 Hypoglycemia and Glucose Levels

The primary outcome will be a study-defined hypoglycemia index computed for the overnight time period (10pm to 7am). This hypoglycemia index is defined as the average amount the glucose is below 70 mg/dL. Glucose levels ≥ 70 mg/dL are assigned a value of zero:

$$\textit{Hypoglycemia index} = \frac{1}{n}\sum_{i=1}^{n} \max\{70 - g_i, 0\}.$$

The index will be calculated separately for sedentary and exercise nights for each subject and compared with a paired t-test. The sample variance of the paired differences will be calculated separately for subjects who had the sedentary visit first and those who had the exercise visit first to account for any period effect. Subjects who fail to complete either visit will be excluded from analysis. If the paired differences do not follow a normal distribution, a square-root transformation or a permutation test may be applied as appropriate.

Binary definitions of overnight (10pm-7am) hypoglycemia also will be evaluated:

- at least one reference glucose ≤ 70 mg/dL.
- at least one reference glucose ≤ 60 mg/dL.
- at least two consecutive reference values drawn within 45 minutes of each other both ≤ 70 mg/dL.
- at least two consecutive reference values drawn within 45 minutes of each other both ≤ 60 mg/dL.
- at least one continuous glucose sensor episode ≤ 70 mg/dL (see below for definition of "episode").
- at least one continuous glucose sensor episode ≤ 60 mg/dL.

For each of these definitions, any treatment for hypoglycemia will count as an event regardless of the measured glucose values.

A continuous glucose sensor episode ≤ 70 mg/dL will be defined as at least two sensor glucose values (not necessarily consecutive) ≤ 70 mg/dL without any intervening values > 80 mg/dL. There may be skips during the episode, but for no more than 30 consecutive minutes. An analogous definition will be used for continuous glucose sensor episodes ≤ 60 mg/dL (two values ≤ 60 mg/dL without any intervening values > 70 mg/dL). For each binary definition, the percentages of subjects with hypoglycemia on sedentary vs. exercise days will be compared using a modified version McNemar's test to account for a possible period effect.

The following continuous outcomes will also be calculated separately on sedentary and exercise nights and analyzed as described above for the primary outcome:

- post-prandial glucose excursion
- overnight low blood glucose index (LBGI)[31]
- mean overnight reference glucose
- percent of reference glucose values in target range 80-160 mg/dL from 6pm to 7am

4.1.1.1 Glucose Changes during Exercise

Descriptive statistics will be computed for the exercise days, overall and stratified according to baseline glucose, for the following:

- Proportion of subjects with a decrease in BG of at least 20%
- Proportion of subjects with a decrease in BG of at least 50%
- Proportion of subjects with a decrease in BG to <60 mg/dL
- Among subjects with at least a 20% decrease in BG, time to nadir
- Distribution of maximum decrease and percent decrease in BG

Box plots will also be constructed for the distribution of change separately at 15, 35, 55 and 75 minutes from the start of exercise.

4.1.2 Hyperglycemia

The following binary definitions will be used for hyperglycemia:

- at least one reference glucose > 300 mg/dL.
- at least two consecutive reference values drawn within 45 minutes of each other both > 300 mg/dL.
- at least one continuous glucose sensor episode > 300 mg/dL (2 values > 300 mg/dL with no intervening values < 290 mg/dL).

Any treatment for hyperglycemia or presence of moderate/large ketones will also count as an event in each of these definitions regardless of measured .

For each binary definition, the percentages of subjects with hyperglycemia on sedentary vs. exercise days will be compared using a modified version McNemar's test to account for a possible period effect.

4.1.3 Glucagon and Epinephrine

The mean overnight (10p.m.-7a.m.) level of glucagon and epinephrine will be calculated for each visit as secondary outcomes. Means from exercise vs. sedentary nights will be compared using the same statistical methods as described above in Section 4.1.1 for the primary outcome.

Changes in glucagon and epinephrine during exercise will be summarized with descriptive statistics and box plots analogous to the methods described for glucose in Section 4.1.1.1. Scatterplots of change in glucose by change in glucagon and change in epinephrine during exercise will also be constructed.

4.1.4 Continuous Glucose Sensor Accuracy

4.1.4.1 Continuous Glucose Sensor

The continuous glucose sensor accuracy during the exercise period will be compared with the accuracy using the same sensor prior to the exercise.

Each reference glucose measurement will be paired with the closest sensor reading in a timeframe appropriate to the frequency of the sensor readings. Reference glucose values used to calibrate the sensor will be excluded from the pairing. The sensor time will be lagged by an appropriate amount to account for any processing delays prior to pairing with the reference glucose.

4.1.4.2 Accuracy Measures

Analyses will be done separately for the continuous sensor and the GWB.

The following difference measures will be calculated for each pair:

- Difference (sensor value – reference value)
- Absolute difference (absolute value of the difference)
- Relative difference (difference divided by the reference value)
- Relative absolute difference (RAD: absolute value of the relative difference)
- ISO criteria (binary: within ± 15 mg/dL if reference glucose ≤ 75 mg/dL; within ± 20% if reference glucose > 75 mg/dL).

Results will be stratified by pre-exercise (exercise visit), during exercise and sedentary visit. These will further broken down by reference glucose level.

Confidence intervals and statistical comparisons will be performed using the bootstrap re-sampling technique to account for correlated observations from the same subject.

4.2 Sample Size Estimation

The sample size has been estimated for the study-defined hypoglycemia index, which is serving as the primary outcome. The index is suspected to deviate from a normal distribution, so simulations were run to estimate power. Overnight glucose data were taken from the DirecNet Inpatient Accuracy study where patients did not exercise during the CRC stay. To mimic the potential effect of exercise, a second night of glucose data was simulated for each subject. The simulated data were made similar to the actual data in terms of mean and standard deviation of glucose and hypoglycemia index. The correlation for mean glucose between the real and simulated data was taken to be 0.3 which resulted in a correlation of 0.1 for the hypoglycemia indices. For each patient, one of the data sets (actual or simulated) was randomly labeled the sedentary night and the other the exercise night. The fixed amount 25mg/dL was subtracted from each glucose value on the "exercise" night and the resulting hypoglycemia indices were compared with the paired t-test. This was repeated for 1,000 simulations to estimate power. Simulations were also run subtracting 30 mg/dL from each glucose value on the "exercise" night.

Mean ± SD values of the hypoglycemia index from these simulations were 0.8 ± 2.3, 2.5 ± 4.3 and 3.0 ± 4.9 on nights with mean exercise effects of 0, 25 and 30 mg/dL, respectively. Results suggest that a sample of 75 subjects would give more than 90% power for a mean exercise effect of 25 mg/dL. Using the square-root transformation on the hypoglycemia index improved power from 3-5% in these simulations.

Note that subjects failing to complete either visit will not be included in the primary analysis (Section 4.1.1) and therefore will not count towards the recruitment goal.

4.2.1 Sample Size Reestimation

An interim analysis is planned for the purpose of evaluating the appropriateness of the sample size. There is considerable uncertainty about the parameters used in estimating the sample size: variance of the hypoglycemia index, frequency of hypoglycemia on the sedentary day, and correlation of the overnight glucose values between the sedentary and exercise days.

After data from both visits have been collected for approximately 35 subjects, the pooled mean hypoglycemia index and variance of the differences (exercise vs. sedentary) will be calculated and

used to reestimate sample size. Results will be presented to the DSMB for consideration in determining whether recruitment of additional subjects is warranted.

REFERENCES

1. Marble, A., R.M. Smith, Exercise in diabetes mellitus. *Arch Intern Med* 58:577-88, 1936

2. Zinman, B., M. Vranic, Diabetes and exercise. *Med Clin North Am* 69(1):145-57, 1985

3. Wasserman, D.H., B. Zinman, Exercise in individuals with IDDM. *Diabetes Care* 17(8):924-37, 1994

4. Peirce, N.S., Diabetes and exercise. *Br J Sports Med* 33(3):161-73, 1999

5. Stratton, R., D.P. Wilson, and R.K. Endres, Acute glycemic effects of exercise in adolescents with insulin-dependent diabetes mellitus. *Phys Sportsmed* 16:150-57, 1988

6. Schiffrin, A., S. Parikh, Accommodating planned exercise in type 1 diabetic patients on intensive treatment. *Diabetes Care* 8(4):337-42, 1985

7. Temple, M.Y., O. Bar-Or, and M.C. Riddell, The reliability and repeatability of the blood glucose response to prolonged exercise in adolescent boys with IDDM. *Diabetes Care* 18(3):326-32, 1995

8. Riddell, M.C., et al., Glucose ingestion matched with total carbohydrate utilization attenuates hypoglycemia during exercise in adolescents with IDDM. *Int J Sport Nutr* 9(1):24-34, 1999

9. MacDonald, M.J., Postexecise Late-Onset Hypoglycemia in Insulin-Dependent Diabetic Patients. *Diabetes Care* 10(5):584-588, 1987

10. King, P., et al., Well-being, cerebral function, and physical fatigue after nocturnal hypoglycemia in IDDM. *Diabetes Care* 21(3):341-45, 1998

11. Biankin, S.A., et al., Target-seeking behavior of plasma glucose with exercise in type 1 diabetics. *Diabetes Care* 26(2):297-301, 2003

12. Mauvais-Jarvis, F., et al., Glucose response to intense aerobic exercise in type 1 diabetes: Maintenance of near euglycemia despite a drastic decrease in insulin dose. [Letter]. *Diabetes Care* 26(4):1316-17, 2003

13. Gerich, J.E., et al., Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* 182(108):171-73, 1973

14. Felig, P., J. Wahren, Fuel homeostasis in exercise. *N Engl J Med* 293(21):1078-84, 1975

15. Amiel, S.A., et al., Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. *N Engl J Med* 316(22):1376-83, 1987

16. Cryer, P.E., Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM. A vicious cycle. *Diabetes* 41(3):255-60, 1992

17. Davis, M.R., M. Mellman, and H. Shamoon, Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* 41(10):1335-40, 1992

18. Davis, S.N., et al., Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. *Diabetes* 46(8):1328-35, 1997

19. Widom, B., D.C. Simonson, Intermittent hypoglycemia impairs glucose counterregulation. *Diabetes* 41(12):1597-602, 1992

20. Heller, S.R., P.E. Cryer, Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes* 40(2):223-26, 1991

21. Jones, T.W., et al., Suppresed epinephrine responses during sleep: a contributing factor to the risk of nocturnal hypoglycemia in insulin-dependent diabetes. *N Eng J Med* 338:1657-1662, 1998

22. Davis, S.N., et al., Effects of antecedent hypoglycemia on subsequent counterregulatory responses to exercise. *Diabetes* 49(1):73-81, 2000

23. Galassetti, P., et al., Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. *Am J Physiol Endocrinol Metab* 280(6):E908-17, 2001

24. Galassetti, P., et al., Effect of antecedent hypoglycemia on counterregulatory responses to subsequent euglycemic exercise in type 1 diabetes. *Diabetes* 52:1761-1769, 2003

25. Bottini, P., et al., Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM: evidence for a nonselective defect. *Diabetes* 46(5):814-23, 1997

26. Schneider, S.H., et al., Impaired adrenergic response to prolonged exercise in type I diabetes. *Metabolism* 41(11):1219-25, 1991

27. McGregor, V.P., et al., Limited impact of vigorous exercise on defenses against hypoglycemia: relevance to hypoglycemia-associated autonomic failure. *Diabetes* 51(5):1485-92, 2002

28. Galassetti, P., et al., Effect of gender on counterregulatory responses to euglycemic exercise in type 1 diabetes. *J Clin Endocrinol Metab* 87(11):5144-50, 2002

29. Purdon, C., et al., The roles of insulin and catecholamines in the glucoregulatory response during intense exercise and early recovery in insulin-dependent diabetic and control subjects. *J Clin Endocrinol Metab* 76(3):566-73, 1993

30. Koivisto, V.A., et al., Fuel and fluid homeostasis during long-term exercise in healthy subjects and type I diabetic patients. *Diabetes Care* 15(11):1736-41, 1992

31. Kovatchev, B.P., et al., Symmetrization of the blood glucose measurement scale and its applications. *Diabetes Care* 20(11):1655-58, 1997