T1D Exchange®

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THE USE OF MINI-DOSE GLUCAGON TO PREVENT EXERCISE-INDUCED HYPOGLYCEMIA IN TYPE 1 DIABETES

Statistical Analysis Plan v. 1.0
Protocol v. 3.0

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Version History

Version Number	Author	Approver	Effective Date	Study Stage	Revision Description
1.0	Stephanie DuBose	Craig Kollman	February 24, 2017	Data collection in progress; no analyses performed	Original version

1.0 Study Overview

This project focuses on the development of a new strategy for the prevention of exercise-associated hypoglycemia using mini-dose glucagon.

The primary objective of the protocol is to determine if the administration of mini-dose glucagon administered subcutaneously just before exercise produces better glucose stability than no adjustments for moderate intensity exercise in patients with T1D. It will also be assessed whether mini-dose glucagon before exercise produces better glucose stability than basal insulin reductions or extra carbohydrate consumption.

Subjects 18 to <65 years old with type 1 diabetes (T1D) for at least 2 years and receiving daily insulin who exercise regularly will be enrolled into the multi-center study. The study is a randomized, 4-way crossover trial.

Each participant will undergo four aerobic exercise sessions (in random order) in the CRC, with different strategies for glucose regulation:

- Control Trial: Fasted exercise, no basal insulin reduction
- Strategy 1: Fasted exercise, basal insulin reduction only (50% reduction in basal rate five minutes before exercise, for the duration of the exercise)
- Strategy 2: Fasted exercise, no basal adjustment + pre-exercise glucose tabs (buccal route-40 grams in total)
- Strategy 3: Fasted exercise, no basal adjustment + pre-exercise mini-dose glucagon (sc)

In all 4 sessions, aerobic exercise will be performed in the fasted state (before a standardized meal) for 45 minutes at ~50-55% of the participant's pre-determined aerobic capacity (determined by VO2 max test at screening visit).

2.0 Sample Size

A target of 16 subjects, with replacement of any participants who do not complete the study, was selected as a reasonable sample size for this feasibility study. Considering the primary outcome of glucose, power was approximated using a t-test. The table below provides power estimates based on a paired t-test comparing glucose during the mini-dose glucagon treatment and glucose during the control treatment.

Power Estimates*

Mean difference (mg/dL)	**Correlation**						
	0.2	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**
20	32%	35%	40%	47%	55%	68%	84%
25	46%	51%	57%	65%	74%	85%	96%
30	60%	66%	73%	80%	88%	95%	99%
35	74%	79%	85%	91%	96%	99%	>99%

*Power was calculated for a sample size of 16 participants with a two-sided alpha of 0.05 and assuming a standard deviation of 40 mg/dL for each treatment. (A previous DirectNet exercise study was considered when estimating standard deviation [Tsalikian 2006].

3.0 Analysis Plan

3.1 Sample collection

Blood samples collected through a venous catheter with plasma glucose measured by an YSI analyzer. Blood samples will be collected at:

- Baseline (at -30, -15, -5, 0 min)
- During exercise (at 5, 10, 15, 25, 35, 45 min)
- In recovery post exercise (at 50, 55, 60, 75 min)
- Regularly following a standardized mixed meal for 90 minutes (at 90, 105, 120, 135, and 165 min)

3.2 Definitions of Time Periods

- Exercise—45 minutes of scheduled exercise, even if participant terminated exercise early
- Early recovery—all time points through 30 minutes post exercise session
- Late recovery—all CGM data through noon the day after each exercise session will be included. Periods of interest during late recovery include the below. Timing of meals indicated on participant log; if log is missing or incomplete, meal timing will be estimated.
    - Standard meal during study (30 minutes post exercise) to standard box lunch provided at discharge
    - Box lunch provided at discharge to dinner (of participant's choice)
    - Dinner to 10:00 PM
    - 10:00 PM to 8:00 AM the day following exercise
    - 8:00 AM to noon the day following exercise

3.3 Primary Outcome

The primary outcome for this study will be glucose, as measured by YSI analysis, during exercise and early recovery.

3.3.1 Primary Analysis

The primary analysis for this study will be a descriptive comparison of glycemic response during exercise and early recovery between the mini-dose glucagon group (strategy 3) and the control group. Summary statistics will be calculated. In the event that exercise is terminated early due to glucose <70 mg/dL and the participant is treated for hypoglycemia (or if participant is treated for hypoglycemia during early recovery [prior to the meal]), the nadir glucose value will be carried forward through the end of early recovery.

Analysis will follow the intent-to-treat principle with each visit allocated to one of the above strategies (Section 1.0) according to the randomization assignment. Analysis will include subjects who completed both the mini-dose glucagon and the control exercise session visits with baseline glucose and at least three YSI glucose measurements during exercise and at least two YSI glucose measurements during early recovery available from each visit.

To obtain a test of significance, a treatment comparison of glucose during exercise and early recovery will be completed using a linear mixed model with repeated measures that accounts for the correlation due to the cross-over design and the correlation due to multiple measures, adjusting for baseline glucose level and any period effect. Regression diagnostics will be performed and if the distribution of residuals is skewed, non-parametric methods and transformations will be considered.

3.4 Secondary Outcomes

The following outcome measures will also be analyzed.

- Occurrence of hypoglycemia (<70 mg/dL) as measured by YSI analysis during exercise or early recovery, at the visit level
- Occurrence of hyperglycemia (≥250 mg/dL) as measured by YSI analysis in the following time periods: during exercise, after exercise in early recovery/prior to meal ingestion and for 90 minutes after the meal (2 total hours after the end of exercise), at the visit level
- Glucose levels after a standardized meal (glucose area under the curve analysis)
- Glucose levels in late recovery (afternoon and overnight following the exercise protocols-CGM analysis)
    - Mean
    - Nadir
    - Area above and below curve threshold (i.e. area above or below 70-180 mg/dL)
    - % of time in range (70-180 mg/dL)
    - % of time below 70 mg/dL
    - Occurrence of hypoglycemia (<70 mg/dL) overnight

For sensor data after an exercise session to be included in the analysis, a subject must have at least 12 hours (i.e., 144 CGM readings) from the window after the exercise session through noon the following day.

3.4.1 Secondary Analyses

The secondary outcomes will largely be examined in a descriptive manner. Summary statistics will be calculated by treatment strategy. For binary variables, percentage of participants developing the outcome, with 95% confidence interval, will be calculated by strategy. Strategy 3 will be compared with each of the other strategies in secondary analyses.

Tests of significance will also be obtained using a 2-sided alpha of 0.05. For binary variables, a generalized linear mixed model with a logistic link function for a binary outcome will be fit to assess differences between strategy 3 and each of the other strategies. The model will be adjusted for baseline glucose and any period effect. For continuous variables, a repeated measures linear regression model will be fit adjusting for period and where applicable the baseline value. One model will be run per outcome measure, with multiple pairwise comparisons from the same model. Regression diagnostics will be performed and if the distribution of residuals is skewed, non-parametric methods and transformations will be considered. No formal correction will be made for multiple comparisons.

3.5 Exploratory Analyses

The following outcome measures may be explored:

- Carbohydrate intake (before, during and for 30 min post exercise)
- HR and ratings of perceived exertion
- Insulin and glucose counterregulatory hormones (glucagon, GH, catecholamines, cortisol)
- Inflammatory cytokines (TNFα, IL-6)
- Metabolites (nonesterified fatty acids, β-hydroxybuterate, lactate)

3.6 Additional Tabulations

- Baseline characteristics
- Number of subjects who did not complete the study and reasons for discontinuation

4.1 Safety

Information from all subjects throughout the study will be used to assess safety.

4.1.1 Adverse Event Definitions

A reportable adverse event is any untoward medical occurrence in a study participant, irrespective of whether or not the event is considered treatment-related, that occurs 12 hours prior, during, or the day after each exercise session, except for hypoglycemia, hyperglycemia, injection-related, and exercise-induced events which are only reported as adverse events when the criteria described below are met.

Hypoglycemic events are recorded as Adverse Events if the event required IV dextrose or IM glucagon to treat.

Hyperglycemic events are recorded as Adverse Events if evaluation or treatment was obtained from a health care provider or if the event involved DKA, as defined by the Diabetes Control and Complications Trial (DCCT) and described below, or in the absence of DKA if evaluation or treatment was obtained from a health care provider or an acute event involving hyperglycemia or ketosis.

Hyperglycemic events are classified as DKA if all of the following are present:

- Symptoms such as polyuria, polydipsia, nausea, or vomiting
- Serum ketones >1.5 mmol/L or large/moderate urine ketones
- Either arterial blood pH <7.30 or venous pH <7.24 or serum bicarbonate <15
- Treatment provided in a health care facility

Injection-related events are recorded as Adverse Events if sufficiently severe that treatment was given.

Exercise-induced events are recorded as Adverse Events if the subject falls during the exercise protocol or develops signs or symptoms of a myocardial infarction, poor perfusion (pallor, cyanosis), angina, pathologic arrhythmia, or another medical condition not expected to occur during or following the exercise.

4.1.2 Safety Analysis

Adverse events will be tabulated by strategy.

- Proportion reporting at least one adverse event
- Proportion with an adverse event thought by investigator to be related to study treatment
- Proportion who stopped study treatment in response to an adverse event
- Total number of adverse events reported
- Number of serious adverse events reported
- Number of non-serious adverse events reported

Adverse events will be tabulated by MedDRA categories with frequencies compared between strategies. Similar diagnostics and models will be performed as described for the efficacy outcomes above.