PROTOCOL TITLE:

Hypoglycemia after exercise in type 1 diabetes: Intranasal naloxone as a novel therapy to preserve hypoglycemia counterregulation

STUDY00000026

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VERSION HISTORY

Version #	Version Date	Summary of Changes	Consent Change?
1		Initial, approved	not applicable
2	09/21/2017	Timing of biospecimens, laboratory results, and questionnaires, clarification of meal schedule	Yes, no re-consent required.
3	12/20/2017	Changes to blood pressure schedule, infusion concentrations, visit location clarification	Yes, no re-consent required.
4	03/02/2018	Add physician letter to recruitment methods	No
5	04/05/2018	Eligibility changes, optional meter/pump download	Yes, verbal re-consent
6	09/10/2018	Additional recruitment methods	No
7	11/01/2018	Inclusion criteria	No
8	12/07/2018	Add option to use potassium chloride	No

TABLE OF CONTENTS

ABBREVIATIONS/DEFINITIONS

- DSMB: Data and Safety Monitoring Board
- DSMP: Data and Safety Monitoring Plan
- Euglycemia: normal blood glucose concentration (60-100 mg/dL)
- Hypoglycemia: low blood glucose (<60 mg/dL)

STUDY SUMMARY

Study Title	Hypoglycemia after exercise in type 1 diabetes: Intranasal naloxone as a novel therapy to preserve hypoglycemia counterregulation
Study Design	Randomized, placebo-controlled, crossover design
Primary Objective	Determine if intranasal administration of naloxone during exercise will be a novel approach to preserve the counterregulatory response to hypoglycemia experienced the next day in individuals with type 1 diabetes
Secondary Objective(s)	Pharmacokinetics and pharmacodynamics of naloxone using a population modeling design to explore dosing for future studies
Research Intervention(s)/ Investigational Agents	Naloxone
IND/IDE #	134687 (exempt)
Study Population	Individuals with Type 1 diabetes
Sample Size (number of participants)	Approximately 30
Study Duration for Individual Participants	Up to 15 weeks

1.0 Objectives

1.1 Purpose:

The overall objective of this project is to determine if the intranasal administration of naloxone during exercise will be a novel approach to preserve the counterregulatory response to hypoglycemia experienced the next day in patients with type 1 diabetes. Exercise induced autonomic failure contributes to the development of impaired awareness of hypoglycemia. Treatments that blunt the consequences of exercise induced autonomic failure, such as preserving the post-exercise counterregulatory response to hypoglycemia, may improve awareness of hypoglycemia. Naloxone, an opioid antagonist, is an extremely promising agent. In healthy volunteers, intravenous administration of naloxone during exercise preserved the counterregulatory response to hypoglycemia the following day (1). In this study, we will extend the clinical applicability by administering intranasal naloxone to individuals with type 1 diabetes. Specifically, we will use a randomized, placebo controlled, crossover design to administer drug or placebo to patients with type 1 diabetes during acute exercise and assess the counterregulatory response to hypoglycemia the following day. The use of intranasal naloxone is a highly innovative aspect of this proposal. Intranasal naloxone translates readily to clinical use and, as demonstrated by our preliminary data, achieves similar plasma drug concentrations as after IV administration.

The specific aims and the hypotheses they address are:

Aim 1: To assess the impact of intranasal naloxone administered during exercise on Day 1 on hypoglycemia induced symptom scores measured on Day 2.

Hypothesis 1: Compared to placebo, intranasal naloxone administered on Day 1 will result in higher symptom scores in response to hypoglycemia on Day 2.

Aim 2: To assess the impact of intranasal naloxone administered during exercise on Day 1 on hypoglycemia induced epinephrine secretion on Day 2.

Hypothesis 2: Compared to placebo, intranasal naloxone administered on Day 1 will result in higher peak epinephrine levels in response to hypoglycemia on Day 2.

Secondary aims include assessment of the tolerability of the treatment and evaluation of the effect of the intervention on glycemic control as measured by continuous glucose monitoring for 7 days after each treatment. In addition, the pharmacokinetics of intranasal naloxone will be characterized and the relationship between plasma drug concentration and response will also be explored. In this proof of principle experiment, we will determine if the administration of intranasal naloxone preserves the counterregulatory response to subsequent hypoglycemia. If it does, future investigation will focus on developing this agent for use in the real world, with experiments designed to identify the minimum dose necessary to achieve the preservation, define the duration of the effect, and examine the ease of usability for patients with type 1 diabetes.

2.0 Background

2.1 Significance of Research Question/Purpose:

Glycemic management and quality of life of patients with type 1 diabetes are adversely impacted by hypoglycemia and its associated fear. Because patients with type 1 diabetes are unable to reduce endogenous insulin secretion and increase glucagon secretion when blood

glucose falls they become critically dependent on the sympathoadrenal response to a fall in glucose to prevent profound hypoglycemia. Unfortunately, the sympathoadrenal response is easily blunted by everyday life experiences such as exercise, as has been shown in both healthy controls (2) and participants with type 1 diabetes (3), leading to impaired awareness of subsequent hypoglycemia. Up to 25% of patients with type 1 diabetes are estimated to have impaired awareness of hypoglycemia (4, 5), which places them at great risk for severe hypoglycemia and potential death. Prevention of impaired awareness of hypoglycemia and reversal of the condition when it occurs requires meticulous avoidance of hypoglycemia, which is nearly impossible in this patient group that experiences one or more episodes of hypoglycemia each week (6).

In particular, there is a critical need to address impaired awareness of hypoglycemia induced by exercise. In exercise induced autonomic failure, the glucose level that triggers the counterregulatory response to hypoglycemia on a day after exercise is lower than the value that triggers the response to hypoglycemia on a day following inactivity. Indeed, the first sign of hypoglycemia in the days following exercise may be neuroglycopenia (7). Many practitioners instruct patients with type 1 diabetes to reduce insulin doses in anticipation of hypoglycemia during and after exercise, but such measures have not be beneficial in reducing hypoglycemia that occurs many hours post activity (8). Therefore, innovative therapies that preserve the counterregulatory response to hypoglycemia experienced post exercise are urgently needed.

2.2 Preliminary Data:

In healthy volunteers, intravenous administration of naloxone during exercise preserved the counterregulatory response to hypoglycemia the following day (1).

2.3 Existing Literature:

Activation of opioid signaling in the brain is believed to be one mechanism that contributes to the development of impaired awareness of hypoglycemia (8). Previous studies have demonstrated that intravenous administration of the opioid antagonist naloxone during exercise prevents the development of exercise associated autonomic failure in humans (1).

Intranasal administration has long been used for therapies like glucocorticoids and has recently become available as an emergency treatment for opioid drug overdose using naloxone, the same drug we will test in this trial. Easy to use and highly portable, a intranasal delivery device that can be used during exercise to administer a drug already shown to prevent exercise induced autonomic failure would greatly expand the options available to patients with type 1 diabetes who want to exercise without putting themselves at risk for impaired awareness of hypoglycemia in subsequent days.

3.0 Study Endpoints/Events/Outcomes

3.1 Primary Endpoint/Event/Outcome:

The primary outcome variable for Aim 1 will be the difference, naloxone vs. saline, in symptom scores collected using a standard questionnaire during the hypoglycemic clamp on Day 2, after administration of intranasal treatment during exercise on Day 1.

The primary outcome variable for Aim 2 will be the difference, naloxone vs. saline, in peak epinephrine levels measured during the hypoglycemic clamp on Day 2, after administration of treatment during exercise on Day 1.

3.2 Secondary Endpoint(s)/Event(s)/Outcome(s):

Secondary endpoints will include:

- Glucose levels will be measured by continuous glucose monitor and home blood glucose monitoring during the 7 days after each experiment with specific levels of interest of > 54 mg/dL, >54mg/dL - <70 mg/dL, and ≤70 mg/dL.
  - Percentage of time of specific ranges
  - Total count of specific ranges
  - Total hypoglycemia symptoms counted during specific ranges

- Plasma naloxone concentrations will be collected to model the pharmacokinetics and pharmacodynamics using a population modeling approach. This will be used to investigate dose levels and regimens for future studies.

4.0 Study Intervention(s)/Investigational Agent(s)

4.1 Description:

This is a single center, single-blind randomized cross over design trial that will compare the impact of intranasal naloxone vs. intranasal saline administration during exercise on day 1 on the response to hypoglycemia on day 2.

Our investigational product is Narcan® intranasal spray, manufactured by ADAPT Pharma, which contains 4 mg naloxone in each metered dose. Naloxone is an opioid antagonist and is FDA approved as both intravenous and intranasal formulations for the complete or partial reversal of opioid depression, including respiratory depression, induced by natural and synthetic opioids.

4.2 Drug/Device Handling:

Investigational Drug Services will handle all drug requirements including randomization and blinding, dispensing, and storage.

4.3 IND/IDE:

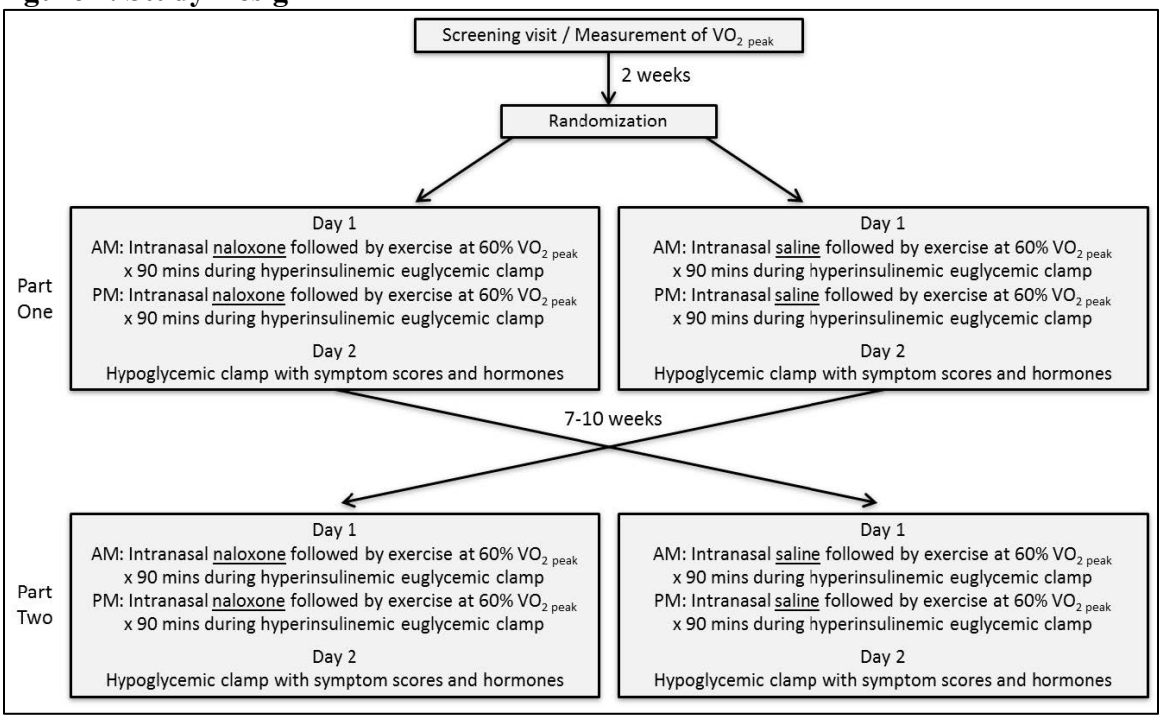
Exempt IND #134687

5.0 Procedures Involved

5.1 Study Design:

This is a single center, single-blind randomized cross over design trial that will compare the impact of intranasal naloxone vs. intranasal saline administration during exercise on day 1 on the response to hypoglycemia on day 2.

Figure 1. Study Design



5.2 Study Procedures:

Screening Visit: At the screening visit, informed consent will be obtained; a standardized form will be used to insure participants meet inclusion/exclusion criteria, and baseline hemoglobin A1c will be collected. Clinical A1c from the medical record can be used if within one month of screening.

Following the collection of blood samples, participants will have their peak aerobic capacity (VO_2peak) determined. Participants will be fitted with a neoprene breathing mask that is connected to an open-circuit spirometry metabolic cart for the collection of expired gas samples. After a warm-up on an electronically-braked bicycle ergometer for 5 minutes, participants will complete a progressive resistance exercise test until volitional exhaustion. VO_2peak will be measured using methodology and equipment already established by our group (9). This measurement will then be used to determine the exercise workload necessary to achieve 60% of VO_2peak during the intervention phases of the study, as was done by Milman (1).

-6 days Part 1 Day 1: In preparation for the intervention visits, participants will be instructed to avoid hypoglycemia in the week before day 1 since that may blunt their response to hypoglycemia during the protocol. They will be asked to avoid exercise, to wear a continuous glucose monitor (provided by the study) during the 6 days before the exercise study and the one day after, and to

monitor and record their blood sugars before meals, at bedtime, and any time they have symptoms of hypoglycemia. Participants will also be given instructions for diabetes management during the days before the study to safely ensure at home insulin has cleared the body by the start of the study.

Participants will be instructed to call the investigators if they have symptomatic hypoglycemia with a glucose of <70 mg/dL or asymptomatic hypoglycemia with a glucose of <54 mg/dL during the 6 days before the exercise studies, after discharge on day 1 or before the hypoglycemic clamp is started on day 2. Participants will be rescheduled if they have episodes of hypoglycemia (defined as symptomatic hypoglycemia and a blood glucose or CGM reading <70 mg/dL or asymptomatic hypoglycemia with a blood glucose of < 54 mg/dL) for more than 20 minutes in the days before Day 1 of the study.

Part 1 Day 1: Participants will be instructed to report for the visit in a fasting state. Before any other research procedures take place, participants will be randomized to receive either naloxone or saline. Intravenous catheters will be placed for the subsequent infusion of 1.0 mU/kg/min insulin, potassium phosphate or potassium chloride (120 mEq/l at 40 cc/hr), and 20% dextrose as needed to maintain euglycemia. When participants reach euglycemia (~ 95 mg/dL), they will be instructed to begin exercise on a stationary bicycle. Adjustments in the exercise workload will be made to assure that participants exercise at a workload requiring 60% of their VO_2peak for 45 minutes based on the VO_2 measured every 15 minutes. They will then be given a 5 minute rest break, followed by another 45 minute period of exercise at 60% VO_2peak. At the start of exercise and 45 minutes after the start of the morning exercise period, participants will be given a 4.0 mg doses of intranasal naloxone or saline in the same volume, according to the randomization assignment.

Participants may be asked to allow a download of their personal glucose meter/pump data. This will be done in research offices using secure software specific for the brand of the glucose meter/pump.

At the completion of the morning exercise, participants will be allowed to rest 3 hours and eat a 15g carbohydrate measured snack. Glycemia will be maintained at euglycemia during this rest period.

At 1 pm, the afternoon period of exercise will start and will be maintained as in the morning for a total of 2 hours. A third dose of 4.0 mg intranasal naloxone or the same volume of saline will be administered at the start of the afternoon exercise, and a final dose of 4.0 mg intranasal naloxone will be given 45 minutes after the start of the afternoon exercise. Blood samples for subsequent measurement of naloxone will be collected at 4-5 predetermined time points from each participant to allow subsequent population pharmacokinetic modeling. After completing the afternoon exercise, participants will be sent home with instruction on avoiding hypoglycemia and using the CGM. Before participants are sent home, a meal will be provided.

Part 1 Day 2: Participants will return the following day, and undergo a two-hour hyperinsulinemic (infusion of 2.0 mU/kg/min) hypoglycemic clamp in which blood glucose will be allowed to drop to 50 mg/dL. Symptom scores will be collected using a standardized questionnaire before and every 15 minutes during the hypoglycemic clamp study. Samples for later measurement of epinephrine, norepinephrine, glucagon and cortisol will be collected at baseline and every 15 minutes during the two-hour infusion period. After completing the clamp, participants will be

given a meal and instructions for using the CGM for the next 7 days. At the completion of Part 1, participants will be scheduled to return to complete part two to receive the alternate therapy.

Wash-out Period: For a total of approximately 7 weeks, participants will be instructed to continue with all normal activities.

Seven days before scheduled Part 2 Visit: Participants will be asked to come in for a visit to apply the CGM. Application of the sensor and instructions are as above.

Part 2 Day 1 and Part 2 Day 2: The second part of the study will follow exactly as above with the exception that the participant will receive the alternate therapy to complete the cross-over. Those who received naloxone in Part 1 will receive saline in Part 2, and vice versa.

A mid-point A1C may be collected at this visit. A clinical A1C from the medical record can be used if within three months of the Screening A1C.

5.3 Follow-Up:

Participants will be contacted at 1 week post final study procedure for adverse event assessment. This can be done by either telephone or email.

	Screening	-6 to -1 days Part 1 Day 1	Part 1 Day 1		Part 1 Day 2	+1 to +7 days Part 1 Day 2	7 Week Washout Period	CGM-Only -7 Days before Part 2	-6 to -1 days Part 2 Day 1	Part 2 Day 1		Part 2 Day 2	+1 to +7 days Part 2 Day 2
			AM	PM						AM	PM		
Consent	X												
A1c	X									X			
VO_{2peak}/bike	X												
Education	X												
Urine Pregnancy Test	X		X							X			
Apply CGM	X							X					
Wear CGM		X	X	X	X	X			X	X	X	X	X
Home blood sugar monitoring		X				X			X				X
Randomization			X										
Download glucose meter/pump			X							X			
Exercise			X	X						X	X		
IV Infusion (insulin, glucose, potassium)			X	X	X					X	X	X	
Naloxone/saline			X[1]	X[1]						X[1]	X[1]		
Blood collection: naloxone concentrations *(only collected during active study drug)*			X[2]	X[2]						X[2]	X[2]		
Blood collection: hormones (epinephrine, norepinephrine, glucagon, cortisol)					X[6]							X[6]	
Glucose measurement			X[5]	X[5]	X[5]					X[5]	X[5]	X[5]	
Blood Pressure	X												
Heart Rate			X[3]	X[3]						X[3]	X[3]		
VO_2			X[4]	X[4]						X[4]	X[4]		
Questionnaires	X				X[7]							X[7]	
Usual Activities							X						
Snack, if necessary			X	X	X					X	X	X	
Meal				X	X						X	X	

[1] Naloxone/saline 4.0mg administered twice, time zero and +30 minutes
[2] Serum collection for naloxone at time points every 20-40 minutes
[3] Heart rate collected continuously
[4] VO_2 collected every 15 minutes
[5] Collected from IV, resulted on bedside Analox machine
[6] Serum collection: hormones
[7] Questionnaires every 15 minutes

6.0 Data and Specimen Banking

6.1 Storage and Access:

Participant's glucose meter/pump data will be downloaded and stored in a de-identified manner on AHC-IS managed servers.

If participants agree, there are two optional parts of this study involving banking. For both the specimen and contact data, only the research study team will have access.

- Blood specimen to be stored indefinitely in secure locked labs. Blood will be stored for future analysis on metabolites to better understand diabetes, hypoglycemia, and basic metabolism. Samples will only be stored with a code and no identifying information.
- Contact information to be stored indefinitely in secure, REDCap™ databases. Information will be used to contact participants about future studies. Data stored will include identifiers, primarily consisting of name, telephone, email, and address.

6.2 Data:

- Glucose meter/pump data will only be stored with a code and no identifying information
- Blood sample will only be stored with a code and no identifying information or other data.
- Future contact stored data will include identifiers, primarily consisting of name, telephone, email, and address for those who indicate they are willing to be contacted in the future.

6.3 Release/Sharing:

No data or specimens will be released or shared.

7.0 Sharing of Results with Participants

Not applicable. Results will not be shared.

8.0 Study Duration

Expected duration for total participation time is approximately 15 weeks.

- Screening Visit: 1 day
- Glucose Monitoring: 6 days
- Part 1, Day 1 and Day 2: 2 days
- Glucose Monitoring: 7 days
- Wash-out period between Part 1 and Part 2: 7 weeks
- Glucose Monitoring: 6 days
- Part 2, Day 1 and Day 2: 2 days
- Glucose Monitoring: 7 days

It is expected to take approximately two and a half years (Y1, Y2, part of Y3) to enroll enough participants to have 28 individuals in matched-pairs complete both parts of the study.

It is expected to take approximately three years (Y1, Y2, Y3) to complete all study procedures on all participants and all final data analysis.

9.0 Study Population

Our study population will be adults with type 1 diabetes between ages 18 and 65 years who have a duration of at least 2 years and who have awareness of hypoglycemia according to the Cox questionnaire (11).

9.1 Inclusion Criteria:

- Type 1 diabetes diagnosed on clinical grounds (history of DKA, use of insulin within 6 months of diagnosis)
- Diabetes duration < 30 years (impaired awareness of hypoglycemia increases with duration so it will be more likely that shorter duration participants will have hypoglycemia awareness) but > 2 years (to ensure that they have lost hypoglycemia induced glucagon secretion as is typical in patients who develop impaired awareness of hypoglycemia)
- Age 18 – 65 years
- Subject reported hemoglobin A1c between 6.8 – 9.0% (range selected to reduce the risk of hypoglycemia and uncontrolled diabetes in the weeks before the study, both of which may affect the responses to hypoglycemia)
    - For subjects on low glucose suspend or closed loop pumps systems A1c can be < 6.8% if a 30 day download of their pump shows that they have had no episodes of hypoglycemia with a glucose < 60 mg/dL for more than 20 min.
- Awareness of hypoglycemia or indeterminate as verified by Cox questionnaire

9.2 Exclusion Criteria:

- History of stroke, seizures (other than those related to hypoglycemia), arrhythmias, active cardiac disease
- History of hypertension or blood pressure > 140/95 mm Hg at screening visit
- Pregnancy or plan to become pregnant during the study period
- Health related limitations in exercise (including but not limited to: angina, uncontrolled asthma, peripheral arterial disease)
- Unwillingness to avoid exercise during the 7 days before each part of the study
- Sedentary life style; defined as less than 60 minutes of moderate exercise per week
- Concomitant medical problems that may prevent the participant from successfully completing the protocol
- Smoking as defined by 2 or more tobacco cigarettes a week
- Daily use of opioids or an opioid antagonist or use in the past two weeks
- History of substance abuse
- Unwillingness to wear a continuous glucose monitor for one week before and one week after each part of the study
- Evidence of autonomic neuropathy; defined as:
    - Early satiety or orthostatic dizziness in the presence of, at least one during the screening visit:
        - Resting heart rate greater than 100
        - Systolic blood pressure fall of greater than 10 mm/Hg when moving from a supine to upright position with no change in pulse

9.3 Screening:

Once the Informed Consent Form and HIPAA authorization has been signed, using a combination of participant self-report and medical record review, research staff will confirm each inclusion and exclusion criteria for eligibility.

10.0 Vulnerable Populations

10.1 Vulnerable Populations:

☐ Children
☐ Pregnant women/Fetuses/Neonates
☐ Prisoners
☐ Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders
☐ Non-English speakers
☐ Those unable to read (illiterate)
☐ Employees of the researcher
☐ Students of the researcher
☒ None of the above

10.2 Additional Safeguards:

Not applicable. Vulnerable populations will not be included in this study.

11.0 Local Number of Participants

11.1 Local Number of Participants to be Consented:

Approximately 30 participants will be enrolled to ensure adequate numbers given possibility for screen failure or loss-to-follow up. Power analysis suggests that 25 participants will be necessary.

12.0 Local Recruitment Methods

12.1 Recruitment Process:

Participants will be directly recruited from the Diabetes and Endocrinology Clinics at M Health. Flyers will be posted at approved locations only with contact information so that interested candidates can call research staff. Flyers with information about the study will also be shared with endocrinologists in the community that they can give their patients about the study. If they are interested, they will directly contact study staff.

In addition, study information including contact information will be posted on ClincalTrials.gov, at the Clinics and Surgery Center using approved recruitment cards, and on social media sites, including but not limited to blogs aimed towards people with Type 1 diabetes, Facebook, and Twitter. In addition, information may be posted on listservs, departmental websites, and in national and local newsletters, and distributed at community events.

Participants in previous studies, who have given permission to be contact, will be called/emailed about the study and asked to consider enrollment. Only participants who have given permission for a direct telephone call will be called, all others will have the letter sent through the post office or electronic mail.

12.2 Source of Participants:

Participants could come from a variety of sources including self-selection from the public population at clinics where flyers have been posted and from medical record review of the patient population.

12.3 Identification of Potential Participants:

Participants could self-identify in response to recruitment advertisements and contact the research staff; at that point, research staff will verbally confirm basic eligibility and schedule a screening visit.

Research staff will also identify potential participants by reviewing medical records of affiliated study endocrinologists. Working with clinic staff, research clinicians will speak with their patients or a letter will be sent to explain the study and provide coordinator contact information. Study health care professionals will also speak to patients if, during the course of a standard-of-care visit, it becomes evident that they might be eligible and/or interested in research. Opt-out status will be reviewed prior to patient contact.

12.4 Recruitment Materials:

Recruitment materials, as submitted to and approved by the IRB include the following:

- Phone script
- Email or letter for former participants matching eligibility
- Letter from affiliated endocrinologist
- Recruitment card for the Clinics and Surgery Center
- Flyer
- Newsletter ad
- Social media guide, ads
- Short study descriptions for newsletters, listservs, etc.

12.5 Payment:

Each participant will be given reimbursements totaling $675 for completing the entire study. This is broken down as follows:

- Screening Visit: $25
- Part 1, Day 1: $150
- Part 1, Day 2: $150
- Part 2, Day 1: $150
- Part 2, Day 2: $200

Compensation will be paid at the completion of each study visit. Compensation will be in the form of a check-request through the University of Minnesota which will be mailed to the participant's house or the Greenphire ClinCard debit card system.

13.0 Withdrawal of Participants

13.1 Withdrawal Circumstances:

Participants will be withdrawn without their consent if at any point following enrollment they develop any of the exclusion criteria.

13.2 Withdrawal Procedures:

No additional data will be collected from a participant who withdraws from the study. Because this is a paired study, withdrawn participants will be replaced with a new participant. Participants recruited to replace a withdrawn participant will be newly randomized and will not be a direct one-to-one replacement following the same treatment sequence.

13.3 Termination Procedures:

Any data collected on early-terminators will still be used, up to the point at which they formally withdrew consent. Baseline data from both completers and non-completers will be used to understand intervention effect and to better design future studies.

14.0 Risks to Participants

14.1 Foreseeable Risks:

Naloxone risks: Potential risks include increased blood pressure, musculoskeletal pain, headache, nasal dryness, nasal edema, nasal congestion, and nasal inflammation. Precipitated withdrawal can occur in those using opioids in the previous 24 hours. Participants will be questioned about opioid use before naloxone/placebo administration.

Bike Ride risks: Discomfort might be felt in body and mind from doing vigorous exercise. There is also risk of injury during exercise testing. These risks are rare and range from minor injuries such as a pulled muscle or sprained ankle to severe injuries such as a heart attack or even sudden death. The exercise test personnel will be monitoring participants every minute of the test to minimize these risks. A study physician or physician's assistant and a study nurse will also be in the room monitoring the subject during the exercise study and prepared to stop the study and administer care should a medical problem develop.

Hypoglycemia risks: Sweating, shakiness, confusion, and feeling "low". Glucose levels will be monitored throughout Part 1 and Part 2 both days and the study will be stopped if participant's levels cannot be monitored due to technical malfunction or study MD/PA feels that it is no longer safe for the participant to continue. Glucose will be immediately be given to normalize the participant's levels.

Blood draw risks: The risks associated with the blood draw include minimal discomfort, and/or bruising. Fainting is possible but unlikely. In very rare cases, a small blood clot can form at the site of the needle insertion. The main inconvenience is the time it will require for the blood to be drawn. The risks of the intravenous line are similar to a blood draw but because it is kept in the vein for a longer period of time (8 hours for Day 1, 4 hours for Day 2), the risk of bruising and discomfort are slightly higher. There will be two intravenous lines placed, one in each arm.

General participation risks: There is always the risk for a breach of confidentiality during the course of any research study.

14.2 Reproduction Risks:

Naloxone is a Schedule C drug, thus its use is not recommended in pregnant women. Animal studies have shown no adverse effect on the fetus but there are no adequate and well-controlled studies in humans so the effect on the human effect is currently unknown. It is also not known if naloxone is excreted in breast milk. Currently pregnant women nor those who are planning on becoming pregnant while enrolled will be excluded.

14.3 Risks to Others:

Not applicable.

15.0 Potential Benefits to Participants

15.1 Potential Benefits:

The only direct benefit to individual participants is a possible better awareness of hypoglycemia symptoms.

16.0 Data Management

16.1 Data Analysis Plan:

All randomized subjects who have complete outcome data for one or both of parts 1 and 2 will be included in the analysis. The Aim 1 primary outcome is difference in symptom scores collected during hypoglycemia on day 2 following the intranasal naloxone vs intranasal saline intervention on day one. The Aim 2 primary outcome will be the peak epinephrine during the hypoglycemic clamp on day 2 of each part, thus each participant will have two observations: one from their naloxone experiment and one from their saline experiment. The statistician will fit a general linear mixed model with fixed effects for treatment (Naloxone vs. saline), treatment order (Naloxone first vs. saline first), and period (Part 1 vs. Part 2) and a random effect for participant. The level of significance will be set at $p< 0.05$.

The study has been confidently designed, with a washout period of 7+ weeks between Parts 1 and 2, to ensure minimization of the possibility of a treatment effect from Part 1 that lingers into Part 2. Summary statistics will be used to examine whether measures of metabolic conditions showed clinically meaningful differences between naltrexone studies and saline studies. If there are, then the treatment comparison will be repeated using the same linear mixed model but with additional

adjustment for the metabolic parameters of concern; this will be done separately for epinephrine and symptoms scores.

Secondary outcomes will be analyzed similarly, using models appropriate for the scale of the outcome (e.g., linear models, logistic models, binomial models).

Data analysis will also consider adjustment for important baseline characteristics, such as age and sex. Diagnostics will be examined to assess model assumptions. Baseline characteristics of those who do complete vs. those who do not complete both parts will be examined and used to inform interpretation of the treatment effect.

16.2 Power Analysis:

The primary outcome variable for Aim 1 will be the difference, naloxone vs. saline, in symptom scores collected using a standard questionnaire during the hypoglycemic clamp on Day 2, after administration of intranasal treatment during exercise on Day 1. The primary outcome variable for Aim 2 will be the difference, naloxone vs. saline, in peak epinephrine levels measured during the hypoglycemic clamp on Day 2, after administration of treatment during exercise on Day 1. In a previous study done with naltrexone (an opioid antagonist that can be given orally), the standard deviation of the differences in symptoms scores collected during hypoglycemia after participants were treated with drug vs. saline was 12.9 points (12). In the preliminary study with naloxone, symptom scores were found to be about 50% greater during hypoglycemia done on Day 2 following administration of intranasal naloxone vs. saline during hypoglycemia on Day 1 (with numerical differences ranging from 7 to 21). Based on these data, it is calculated that the necessary sample size of 25 completers to have 80% power to detect a difference of 7.53 points in symptom scores between those treated with intranasal naloxone vs. saline during exercise on day 1 with $p < 0.05$. Analysis of the data published in Milman et al (1), suggests that at least 15 subjects must complete the study for the power to detect a mean paired difference in hypoglycemia induced epinephrine secretion on Day 1 between those treated with intranasal naloxone vs. saline during exercise on day 1. Therefore, the plan is to enroll 30 subjects to ensure that we complete studies in the 28 subjects we need to address the first aim.

16.3 Data Integrity:

The PI will periodically review data for completeness and to ensure that all procedures are being followed as detailed in the protocol. The assigned Regulatory Specialist will also perform periodic quality assurance monitoring at any point requested by the PI.

17.0 Confidentiality

17.1 Data Security:

All standard confidentiality procedures will be observed for this study and all research staff will be trained before they will be allowed contact with participants or data. All paper data, source or CRF or otherwise, will be kept in locked research offices. Electronic data will be kept in password-protected files on the (N:) drive (\\med.ahc.umn.edu\med).

All University of Minnesota confidentiality and privacy policies and procedures will be followed by all research staff.

CRF data for this study will be entered into a REDCap database, which uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server are housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

Consent documents will not be placed in the participant's medical record. Documentation of research involvement will not be recorded in the medical record.

18.0 Provisions to Monitor the Data to Ensure the Safety of Participants

18.1 Data Integrity Monitoring.

While there will be no planned interim data analysis, the PI will periodically review data for completeness and to ensure that all procedures are being followed as detailed in the protocol. The assigned Regulatory Specialist or CTSI monitor will also perform periodic quality assurance monitoring at any point requested by the PI or at least annually.

18.2 Data Safety Monitoring.

An unblinded Data and Safety Monitoring Board (DSMB) will be assembled, including the following individuals:

- Independent monitor: endocrinologist with clinical and research experience.
  - Brandon Nathan, MD. Associate Professor. Division of Pediatric Endocrinology. Department of Pediatrics, University of Minnesota
- Independent monitor: endocrinologist with clinical and research experience.
  - Melena Bellin, MD. Associate Professor of Pediatric Endocrinology Department of Surgery, University of Minnesota
- Study monitor: statistician with research experience
  - Lynn Eberly, PhD. Associate Professor Division of Biostatistics, School of Public Health, University of Minnesota

The DSMB will review any incidence of early stopping of the study and any incidence of an adverse or serious adverse event at time points of post-5, 10, 15 randomizations and at the final randomization. If at any point, during Part 1 or Part 2, the study was stopped early and an adverse or serious adverse event was reported, the DSMB will review that participant's

data within two weeks. The DSMB will be informed of all adverse and serious adverse events within 48 hours and other events within 7 days. The DMSB may recommend termination of the study at any time based on safety concerns.

The Principal Investigator will oversee the safety of the study, including careful assessment and appropriate reporting of adverse events. All adverse and serious adverse events will be discussed with the PI within 48 hours for standard assessments (causality, expectedness, severity, and seriousness). IRB and other regulatory reporting requirements will be reviewed by study staff and followed with assistance of the Regulatory Specialist assigned to the study.

Specific safety data to be monitored includes those associated with naloxone administration and hypoglycemia. Participants will be continuously monitored during the administration of the naloxone/placebo by study personnel. At each study visit following consent, participants will be asked about their health since the last time they were seen by the study staff.

- Those specifically related to naloxone: muscoskeletal pain, headache, nasal symptoms, blood pressure, and other cardiac events.
- Glucose levels: if at any point during Part 1 or Part 2, glucose monitoring becomes impossible because of technical failure or other issue or if the MD/PA present suspects an immediate concern, the study will be immediately halted and blood sugar given to participant.

19.0 Provisions to Protect the Privacy Interests of Participants

19.1 Protecting Privacy:

Participants will sign a consent form and a HIPAA Authorization which will detail what data and under what circumstances will be shared with research staff and non-research staff.

It is not anticipated that any survey will ask intrusive or difficult questions. However, surveys will be explained in full detail in the consent so that participants can make informed decisions before they enroll. The consent will also detail under which conditions any information may be shared with those outside of the internal research staff.

19.2 Access to Participants:

Participants will sign a consent and HIPAA authorization.

20.0 Compensation for Research-Related Injury

20.1 Compensation for Research-Related Injury:

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to the subject or their insurance company.

21.0 Consent Process

21.1 Consent Process (when consent will be obtained):

At the start of the screening visit, informed consent will be obtained by the research coordinator. The entire consent document will be reviewed, including all study procedures and expectations, risks, benefits, and what volunteering means. Candidates will be given time to read the consent, ask questions, and to take the consent home to review if requested. All consent procedures will take place in a private room at the Clinical Research Unit at the University of Minnesota. During the study, and especially before naloxone/saline administration, specific risks and procedures will be reviewed to ensure continued voluntary and informed consent.

21.2 Waiver or Alteration of Consent Process (when consent will not be obtained):

Not applicable.

21.3 Non-English Speaking Participants:

Not applicable.

21.4 Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age):

Not applicable.

21.5 Cognitively Impaired Adults, or adults with fluctuating or diminished capacity to consent:

Not applicable.

21.6 Adults Unable to Consent:

Not applicable.

22.0 Setting

22.1 Research Sites:

All participant-facing research procedures will take place at the Clinical Research Unit or Delaware Clinical Research Unit at the University of Minnesota, including consent, screening, and Part 1 and Part 2. All procedures will take place in private exam or consulting rooms. The second CGM placement may also take place at the Clinic and Surgery Center (CSC) or in research space in PWB.

23.0 Multi-Site Research

Not applicable.

24.0 Resources Available

24.1 Resources Available:

Approximately 30 participants are expected to consent to the study to ensure 25 matched-pairs completing the study. Over the assumed two and a half years needed for recruitment, this is about 2-3 participants per month. Previous, similar studies have been able to recruit this number and the enrollment rate is expected to be similar given the patient population of Diabetes and Endocrinology clinics at M Health where most recruitment flyers will be posted.

Study visits will be completed at the Clinical Research Unit or Delaware Clinical Research Unit as contracted with CTSI, the Clinic and Surgery Center (CSC), or research space within the Phillips-Wangensteen Building. Staff has private offices for non-participant research activities, primarily located in the Phillips-Wangensteen Building.

Participants will be referred to an endocrinologist or their personal physician for any medical or psychological evaluation that may be required as a result of the research.

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