

Clinical Study Protocol

Protocol Title:

A randomized, three-way, crossover study to assess the efficacy of fast-acting insulin-plus-pramlintide closed-loop co-administration, regular insulin-plus-pramlintide closed-loop co-administration, and fast-acting insulin-alone closed-loop infusion in regulating glucose levels over a 24-hour period in adults with type 1 diabetes in inpatient settings.

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1 Background and rationale

Amylin is a gluco-regulatory beta-cell hormone that is co-secreted with insulin in response to nutrient stimuli, and is deficient in patients with type 1 diabetes. Amylin, in the postprandial period, contributes to regulating glucose levels by delaying gastric emptying, suppressing nutrient-stimulated glucagon secretion, and increasing satiety. Pramlintide is a synthetic analog of the hormone amylin.

Co-injection of pramlintide with insulin at meal times improves glucose control [1] and reduces HbA1c levels [2], and these improvements occur with a concomitant reduction in insulin dosing [1, 3]. Despite its established benefits, pramlintide is under-utilized, likely because it is an injectable medication that requires one injection per meal.

Co-formulation of insulin and pramlintide that alleviates the need of extra injections might increase the uptake of pramlintide therapy. Moreover, in pump-treated patients, administration of insulin and pramlintide co-formulation during fasting as well as prandial conditions better mimics the natural physiology of the pancreas, and may prove more effective than administering pramlintide at meal times only [4].

Recent advances of glucose sensors have motivated the research towards closed-loop delivery systems to automatically regulate glucose levels. The closed-loop system is composed of three components: a glucose sensor, an infusion pump, and a dosing algorithm to control how much insulin is delivered based on the sensor readings. Although insulin-alone closed-loop systems significantly improve glucose control compared to conventional pump therapy, they still do not normalize postprandial glucose levels. Patients treated with current insulin-only closed-loop systems maintain glucose levels in the target range 70-75% of the time, but spend 20-25% (i.e., 5-6 hours per day) of the time in hyperglycemia, particularly postprandially [5].

A closed-loop system that delivers both insulin and pramlintide, based on glucose sensor readings, has the potential to better normalize glucose levels. Weinzimer et al. [6] studied the acute effect of pramlintide on the performance of closed-loop delivery, and found that adding pramlintide to the closed-loop system improves glucose control, especially postprandially. Sherr et al. [7] analyzed the chronic effect of pramlintide given as a prandial bolus, and observed that adding pramlintide to the closed-loop system had a 39% reduction in peak postprandial plasma glucose levels, a significant delay in the time to peak plasma glucose level as well as overall a significantly greater time in target levels (3.9 - 10 mmol/L) during daytime hours. In both these studies, pramlintide was delivered at meal times only. In this protocol, we aim to assess the efficacy of the simultaneous, closed-loop, basal-bolus

infusion of pramlintide and insulin with a fixed (i.e., mimicking co-formulation) in controlling glucose levels.

Co-administration of pramlintide at mealtimes along with rapid-acting insulin may lead to an immediate post-prandial plasma sugar reduction followed by a delayed hyperglycemia [8]. The decrease in blood sugar levels in the early postprandial period are due to the faster action time of pramlintide combined with the fast action of rapid-acting insulin. Literature data suggests that the pharmacodynamics of regular insulin may better match the effect of pramlintide compared to the pharmacodynamics of fast-acting insulin (Figure 1). Moreover, the cost of regular insulin is significantly lower than fast-acting insulin. Therefore, if a similar (or better) glucose profile can be achieved with regular insulin-plus-pramlintide compared to fast-acting insulin-plus-pramlintide, then a co-formulation employing regular insulin should be prioritized.

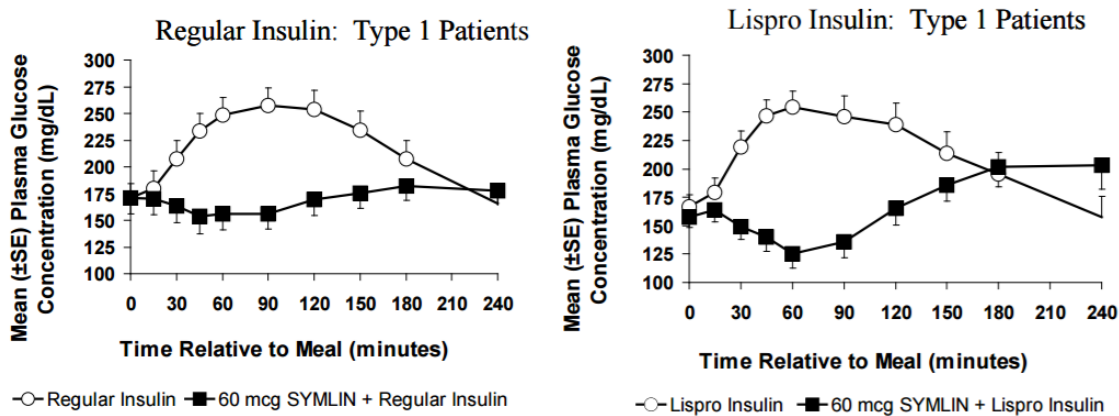


Figure 1 Postprandial glucose profiles in type 1 diabetes following injections of regular insulin (left) or insulin lispro (right) plus either placebo or 60 μ g pramlintide bolus at meal (breakfast) time. Reprinted from the Symlin label [9].

Therefore, in this protocol, we aim to assess the effect of the simultaneous, closed-loop, basal-bolus infusion of pramlintide with insulin at a fixed ratio in controlling glucose levels. In the first experimental arm, we propose to infuse pramlintide with fast-acting insulin. In the second experimental arm, pramlintide will be infused with regular insulin. The control arm will be fast-acting insulin-alone closed-loop system.

2 Trial Objectives

The aim of the study is to assess the efficacy of the simultaneous, closed-loop, basal-bolus infusion of pramlintide with fast-acting insulin at a fixed ratio and pramlintide with regular insulin at a fixed ratio in controlling glucose levels compared to fast-acting insulin-alone closed-loop infusion. Pramlintide will be initiated for a minimum of 10 days with a target of 14 days before the closed-loop experiment,

which will mimic more closely the performance of the system during the chronic use of pramlintide rather than its acute use. Regular insulin will replace participant's usual insulin and will be initiated for a minimum of 10 days with a target of 14 days before the closed-loop experiment.

3 Hypothesis

Primary hypotheses:

1. During closed-loop control, the simultaneous basal-bolus infusion of pramlintide and fast-acting insulin improves glucose control compared to fast-acting insulin-alone infusion.
2. During closed-loop control, the simultaneous basal-bolus infusion of pramlintide and regular insulin improves glucose control compared to fast-acting insulin-alone infusion.

4 Study Design

4.1 Trial Design

This study is a randomized, three-way, crossover trial to compare the efficacy of **i)** fast-acting insulin-plus-pramlintide closed-loop delivery, **ii)** regular insulin-plus-pramlintide closed-loop delivery, and **iii)** fast-acting insulin-alone closed-loop delivery in regulating glucose levels over a period of 24 hours in a study on adults in inpatient settings. Insulin (fast-acting and regular) and pramlintide are given with fixed ratio (6 µg of pramlintide for each unit of insulin).

Before each 24-hour intervention visit, the participant's insulin therapy (basal rates and insulin-to-carbohydrate ratios) will be optimized for a minimum of 10 days, with a target of 14 days. If a participant is randomized to either rapid or regular insulin-plus-pramlintide arms, pramlintide will be infused using a second pump at a fixed ratio for a minimum of 10 days, and insulin therapy will be optimized. Regular insulin will replace participant's usual insulin and will be initiated for a minimum of 10 days, and insulin therapy will be optimized.

There will be a wash-out period of 0 to 42 days between the three intervention arms (termination of 24-hr intervention and start of next optimization period).

4.2 Study Population

The trial aims to enroll adult participants with type 1 diabetes using insulin pump therapy.

4.2.1 Inclusion criteria

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

1. Males and females \geq 18 years of age.
2. Clinical diagnosis of type 1 diabetes for at least 12 months.
The diagnosis of type 1 diabetes is based on the investigator's judgment; C peptide level and antibody determinations are not needed.
3. Insulin pump therapy for at least 6 months.
4. HbA1c \leq 10%.

4.2.2 Exclusion criteria

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

1. Current or \leq 1 month use of other antihyperglycemic agents (SGLT2, GLP-1, Metformin, Acarbose, etc...).
2. Severe hypoglycemic episode within one month of admission.
3. Severe diabetes keto-acidosis episode within one month of admission.
4. Planned or ongoing pregnancy.
5. Known or suspected allergy to the study drugs.
6. Gastroparesis.
7. Use of prokinetic drugs that stimulate gastric emptying (domperidone, cisapride, metoclopramide).
8. Clinically significant nephropathy, neuropathy or retinopathy as judged by the investigator.
9. Recent ($<$ 6 months) acute macrovascular event e.g. acute coronary syndrome or cardiac surgery.
10. Current use of glucocorticoid medication.
11. Other serious medical illness likely to interfere with study participation or with the ability to complete the trial by the judgment of the investigator.
12. Failure to comply with team's recommendations (e.g. not willing to eat meals/snacks, not willing to change pump parameters, etc.).

4.3 Study Interventions

4.3.1 Fast Acting Insulin-alone closed-loop delivery

Variable subcutaneous insulin will be delivered to regulate glucose levels. Participants' usual fast-acting insulin (aspart, lispro, or glulisine) will be infused using a subcutaneous insulin infusion pump (Minimed® Veo™ or 630G, Medtronic). The participant will be wearing up to 2 glucose sensors, including Dexcom G4® or G5® (mandatory), and Abbott FreeStyle Libre (optional). The glucose level as measured by the Dexcom glucose sensor (Dexcom G4® or G5®, Dexcom, Inc.) will be entered manually into the computer every 10 minutes. The pumps infusion rates will then be changed manually based on the computer-generated recommendation. The computer-generated recommendations are based on a dosing algorithm [5]. Predictive algorithms have been successfully used in closed-loop studies in children, adolescents, pregnant women and adults [5]. The carbohydrate content for every

ingested meal will be entered into the algorithm to calculate the insulin prandial bolus. The carbohydrate content will be entered at the onset of the meal.

4.3.2 Fast-Acting Insulin-plus-pramlintide closed-loop delivery

Variable subcutaneous insulin and pramlintide will be delivered at a fixed ratio (6 µg pramlintide/unit insulin) to regulate glucose levels. Participants' usual fast-acting insulin (aspart, lispro, or glulisine) will be infused using a subcutaneous insulin infusion pump (Minimed® Veo™ or 630G, Medtronic). Basal-bolus pramlintide (AstraZeneca) will be infused using a second pump (Minimed® Veo™ or 630G, Medtronic). The participant will be wearing up to 2 glucose sensors, including Dexcom G4® or G5® (mandatory), and Abbott FreeStyle Libre (optional). The glucose level as measured by the Dexcom glucose sensor (Dexcom G4® or G5®, Dexcom, Inc.) will be entered manually into the computer every 10 minutes. The pumps' infusion rates will then be changed manually based on the computer-generated recommendation, while still maintaining the 6 µg/unit ratio. The computer-generated recommendations are based on a dosing algorithm [5]. The carbohydrate content for meals will be entered into the algorithm to calculate the prandial boluses for insulin. The carbohydrate content will be entered at the onset of the meal.

4.3.3 Regular Insulin-plus-pramlintide closed-loop delivery

Variable subcutaneous insulin and pramlintide will be delivered at a fixed ratio to regulate glucose levels. Regular insulin (Humulin R) will be infused using a subcutaneous insulin infusion pump (Minimed® Veo™ or 630G, Medtronic). Basal-bolus pramlintide (AstraZeneca) will also be infused using a second pump (Minimed® Veo™ or 630G, Medtronic). The participant will be wearing up to 2 glucose sensors, including Dexcom G4® or G5® (mandatory), and Abbott FreeStyle Libre (optional). The glucose level as measured by the Dexcom glucose sensor (Dexcom G4® or G5®, Dexcom, Inc.) will be entered manually into the computer every 10 minutes. The pumps' infusion rate will then be changed manually based on the computer-generated recommendation, without maintaining any specific infusion ratio of the two hormones. The computer-generated recommendations are based on a dosing algorithm [5]. The carbohydrate content for meals will be entered into the algorithm to calculate the prandial boluses for insulin.

5 Study Procedures and Visit Schedule

5.1 Recruitment

Potential participants will be recruited at the McGill University Health Centre - Adult Diabetes Clinic, Montréal, Canada. A total of 28 participants will be enrolled in the study. The study will be conducted at the Centre for Innovative Medicine (CIM) at McGill University Health Centre Research Institute,

Montréal, Canada. Participants showing interest in participation will have the study fully explained to them and will be offered the opportunity to ask questions. Interested participants that meet basic eligibility criteria will be scheduled for the admission visit.

5.2 Visit Schedules

5.2.1 Admission Visit (Visit 1)

At the admission visit, the following procedures will be taken:

- Participants will sign the consent form.
- Inclusion and exclusion criteria will be assessed
- A medical visit will be taken to establish medical history (i.e. recent severe hypo- and hyperglycemia episodes, micro- and macrovascular complications, comorbidities and precise list of all medications).
- Records of current insulin therapy (total daily dose, carbohydrate to insulin ratios, basal rates, insulin sensitivity, target blood glucose levels and active insulin time).
- A1c level if no recent (\leq 1 month) result is available.
- For women for childbearing potential, a pregnancy test will be performed
 - Women 50 years or older with an absence of menstrual bleeding for the past year will not be required to take the pregnancy test.
 - Women that underwent hysterectomy will not be required to take the pregnancy test.
- Serum creatinine and eGFR level if no recent (\leq 1 month) results are available.
- Weight and height measurements will be obtained.
- Participants will complete a questionnaire on the awareness of hypoglycemia.

5.2.2 Initiation Optimization Visit (Visit 2)

At initiation optimization visit, the following procedures will be taken:

- Participant will receive training on the study material (glucose sensor, insulin pump and artificial pancreas).
- Any question concerning the optimization period or the intervention visit will be answered.
- Indications regarding the use of regular insulin and pramlintide will be given to the participant.
- If the participant is using a pump other than Medtronic, the participant will receive a study pump (Minimed® Veo™ or 630G, Medtronic) to infuse rapid insulin and regular insulin. The patient may switch to the study pump for the optimization period or continue using their own pump. The patient will be responsible for making the switch to the study pump on their own, at least 1 day prior to the intervention.

- Participants will receive pramlintide, second pump (Minimed® Veo™ or 630G, Medtronic) and second catheter for infusion of pramlintide. Infusion of pramlintide will be initiated as per the optimization schedule, and may require the patient to initiate it on their own, outside of visit 2.
- A glucose sensor (Dexcom G4® or G5®, Dexcom, Inc.) will be provided. The patient may be required to insert the sensor on their own, outside of visit 2, as per the optimization schedule.
- Optionally, if the patient consents to wearing two glucose sensors, an additional sensor (FreeStyle Libre, Abbott) will be provided by the research team and training on the sensor use will be provided by a trained research member.
 - Installation of FreeStyle Libre sensor will take place either **i)** 0-2 days **ii)** 6-8 days or **iii)** 11-13 days prior to the intervention. The sensor installation schedule will be determined by a member of the research team.

After this visit, the treatment optimization for a minimum of 10 days will be conducted.

5.2.3 Intervention visits (Visit 3, 4 and 5)

After the optimization period, each study participant will be admitted to our clinical research facility for a 24-hour intervention. Details of intervention visits are presented in subsequent sections.

5.3 Randomization

Each study participant will be assigned a unique anonymous identification number, which will be used throughout the study. A blocked balanced randomization will be used to determine the order of the interventions.

Participants will be randomized to either **i)** fast-acting insulin-plus-pramlintide closed-loop delivery, **ii)** regular insulin-plus-pramlintide closed-loop delivery, and **iii)** fast-acting insulin-alone closed-loop delivery.

Randomization envelopes will be opened after admissions visit 1.

5.4 Treatment Optimization

The optimization period will last for a minimum of 10 days, with a target of 14 days. During this optimization period, each participant will wear a glucose sensor. A research team member will contact (by phone or email) study participants in order to optimize their treatment parameters (insulin to carbohydrate ratios, basal rates, etc.). The insulin dose will be titrated to meet target glucose according to the continuous glucose sensor. Research team might ask study participants to present in person if deemed necessarily for treatment optimization. In addition, gastrointestinal symptoms (nausea, vomiting, bloating, and heart burn) will be evaluated during the optimization period, and during the

inpatient interventions. Intervention visits will be conducted after this optimization period. Similar contact and therapy optimization procedure will be applied prior to each intervention.

During days when participant will be wearing the CGM sensor he should be advised to avoid consumption of drugs containing acetaminophen due to possible effect on accuracy of glucose readings. If acetaminophen is part of the subject's standard therapy, the study physician will discuss alternative treatments with the participant.

5.5 Pre-study Test Participants

Up to 6 participants will be analysed to optimize study design and procedures. The participants will partake in the three arms, including the optimization periods. Data from these participants will not be included in the final study analysis.

6 Intervention Visits Procedures

6.1 24-hour Intervention Procedures

For patient's convenience, the patient will be given an option to sleep on site one night prior to the intervention, with research staff available on site. Study procedures, however, will start in the morning of the scheduled intervention.

Identical procedures will be used during the three 24-hour inpatient visits. Each 24-hour intervention visit will include three meals (8:00, 12:00, and 17:00), evening snack (21:00), and an overnight stay. Meals and snacks will be standardized between visits for the same participant. Participants will be admitted at the research facility around 7:00 and will be discharged between 8:00 and 8:30 the next morning. For the intervention visit with regular insulin and pramlintide, the visit will start at 7:40am.

Insulin (fast-acting or regular) infusion will be changed manually every 10 minutes based on the control algorithm that relies on the glucose sensor readings. The basal-bolus pramlintide will be infused using the second pump with a fixed ratio to insulin delivery (i.e., will also be changed manually every 10 minutes). Participants will be blinded to the glucose sensor measurements. Venous blood samples will be sampled for the measurement of plasma glucose, plasma insulin, plasma glucagon, and plasma amylin.

The detailed study procedures description is as follows.

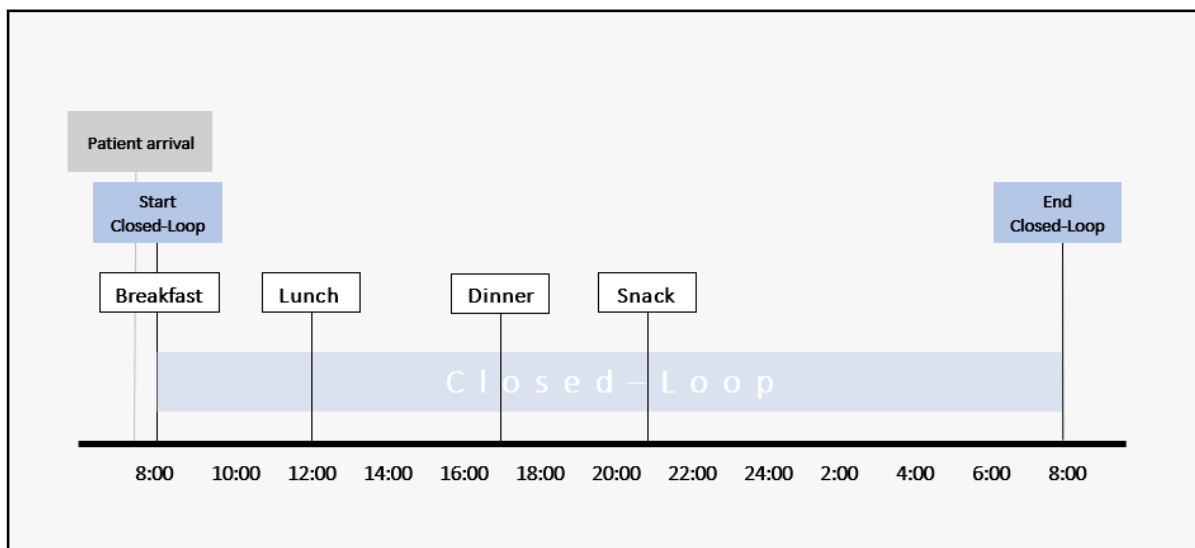


Figure 2 Study intervention procedures

6.1.1 Initial procedures (7:00-8:00)

- Participants will be admitted at the MUHC Research Institute Centre for Innovative Medicine (CIM) around 7:00.
- Participant's weight will be obtained.
- For women for childbearing potential, a pregnancy test will be performed
 - Women 50 years or older with an absence of menstrual bleeding for the past year will not be required to take the pregnancy test.
 - Women that underwent hysterectomy will not be required to take the pregnancy test.
- Revision of inclusion and exclusion criteria
- Revision of the patient's medication
- A cannula will be inserted into the antecubital or hand vein for blood sampling purposes.
- Participants will be asked to calibrate the glucose sensor using their capillary glucose level as measured by their meter.
- Presence of gastrointestinal symptoms (nausea, vomiting, bloating, and heart burn) will be assessed in the fasting state.
- The closed-loop study will begin at 7:40 (regular insulin) or 8:00 (rapid insulin).

6.1.2 Procedures related to Breakfast (8:00)

At 8:00, a mixed meal will be served containing 30g – 60g of carbohydrates. The carbohydrate content will be entered at the onset of the meal. Participants will have a choice to personalize the content of their meal from a menu of two main options. Study participants will consume the same meal at each

visit. Presence of gastrointestinal symptoms (nausea, vomiting, bloating, and heart burn) will be assessed in the post-prandial period.

6.1.3 Procedures related to Lunch (12:00)

At 12:00, a mixed meal will be served containing 50g – 80g of carbohydrates. The carbohydrate content will be entered at the onset of the meal. Participants will have a choice to personalize the content of their meal from a menu of four main options. Study participants will consume the same meal at each visit. Presence of gastrointestinal symptoms (nausea, vomiting, bloating, and heart burn) will be assessed in the post-prandial period.

6.1.4 Procedures related to Dinner (17:00)

At 17:00, a mixed meal will be served containing 70g – 100g of carbohydrates. The carbohydrate content will be entered at the onset of the meal. Participants will have a choice to personalize the content of their meal from a menu of four main options. Study participants will consume the same meal at each visit. Presence of gastrointestinal symptoms (nausea, vomiting, bloating, and heart burn) will be assessed in the post-prandial period.

6.1.5 Night procedures (21:00-8:00)

A 20g – 40g carbohydrate bedtime snack will be given at 21:00. The carbohydrate content will be entered at the onset of the meal. Participants will have a choice to personalize the content of their meal from a menu of 6 options. Study participants will consume the same snack at each visit. Presence of gastrointestinal symptoms (nausea, vomiting, bloating, and heart burn) will be assessed in the post-prandial period.

6.1.1 Discharge procedure (8:00 to 8:30 next morning)

Participants will be discharged between 8:00 and 8:30 the next morning from the inpatient admission. If patient's plasma or capillary glucose at discharge is below 5mmol/L, the patient will be offered a snack (oral carbohydrates 10-40 gCHO).

6.1.2 Procedures related to Activity

Throughout the 24-hour intervention, participants will be allowed to perform light activities such as walking, and play card, board, and video games. Participants will be encouraged to walk at least 30 min per day. If the participant's walk, the same activity should be performed at the three intervention visits.

6.2 Sensor Measurement

The algorithm requires to be updated with the current sensor measurement every 10 minutes from the start of the study until the end of the study. While the aim is to strictly follow the schedule, any situations that delay or disable the research staff from taking the sensor measurement and/or updating the pump settings on time will be recorded in patient's Case Report File, but will not be considered a protocol deviation.

6.3 Blood Sampling

6.3.1 Blood sampling schedule

Blood samples (2 ml) will be obtained according to the following schedule:

8:00, 8:10, 8:20, 8:30, 8:40, 9:00, 9:20, 9:40, 10:00, 10:30, 11:00, 11:30, 12:00, 12:10, 12:20, 12:30, 12:40, 13:00, 13:20, 13:40, 14:00, 14:30, 15:00, 15:30, 16:00, 16:30, 17:00, 17:10, 17:20, 17:30, 17:40, 18:00, 18:20, 18:40, 19:00, 19:30, 20:00, 20:30, 21:00, 21:30, 22:00, 22:30, 23:00, 23:30, 0:00, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 6:30, 7:00, 7:30, 8:00.

Blood samples (2ml) will be obtained according to the following schedule for the intervention with regular insulin and pramlintide:

7:40, 7:50, 8:00, 8:10, 8:20, 8:40, 9:00, 9:20, 9:40, 10:00, 10:30, 11:00, 11:30, 11:40, 11:50, 12:00, 12:10, 12:20, 12:40, 13:00, 13:20, 13:40, 14:00, 14:30, 15:00, 15:30, 16:00, 16:30, 16:40, 16:50, 17:00, 17:10, 17:20, 17:40, 18:00, 18:20, 18:40, 19:00, 19:30, 20:00, 20:30, 21:00, 21:30, 22:00, 22:30, 23:00, 23:30, 0:00, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 6:30, 7:00, 7:30, 8:00.

In addition, if plasma glucose is < 3.3 mmol/L, extra samples may be taken each 10-15 minutes until plasma glucose rises again above 3.3 mmol/L, then the pre-defined sampling schedule will be re-followed.

Any situations that delay or disable the nurse from taking the plasma sample on time will be recorded in patient's Case Report File, but will not be considered a protocol deviation. Additionally, any extra plasma samples will be recorded in patient's Case Report File, but will not be considered a protocol deviation.

In the event that the plasma glucose is unavailable due to IV issues or for other reasons, the study will proceed based on sensor glucose values.

To reduce costs, only a sub-group of participants (n=14) may be analyzed to measure plasma insulin, plasma glucagon and plasma amylin concentrations. Samples for the other 14 participants may be analyzed if deemed necessary by the investigators based on the availability of funds. Samples that are measured will be chosen in a scientifically appropriate manner.

Plasma glucose will be measured in real time using YSI2300 STAT Plus Analyser (Yellow Springs, Ohio, USA). Plasma insulin, amylin and glucagon will be measured by immunoassay (Millipore, Billerica, MA, USA).

6.3.2 Blood volume

Blood volume drawn will be: 61 samples x 2 ml = 122 ml for intervention visits of rapid insulin alone and rapid insulin and pramlintide.

Blood volume drawn will be: 64 samples x 2ml = 128ml for the intervention visit of regular insulin and pramlintide.

Note that extra samples might be drawn as required.

6.4 Intervention Criteria

6.4.1 Treatment of Hypoglycemia

Hypoglycemia is very common in real life in patients with type 1 diabetes. The JDRF CGM trial reported hypoglycemia <3.3 mmol/L in about 9% of the nights, half of them lasting at least one hour and quarter of them lasting at least two hours [10]. Patients also experience symptomatic hypoglycemia around 2.7 times per week [11]. Moreover, hypoglycemia remains underestimated with up to 60% of events remaining unrecognized [12]. Therefore, ideally, we would like to observe the differences in hypoglycemia between the interventions, especially that hypoglycemia is identified as the major limiting factor to achieve glycemic goals [13]. This can be best achieved if the investigators do not intervene during the study, replicating home settings as much as possible. However, despite the importance of observing hypoglycemia events and their duration as they would happen spontaneously in real life, research staff will need to intervene in the event of hypoglycemia for safety reasons.

By default, treatment of hypoglycemia will be based on plasma glucose levels. However, in the event plasma glucose measurements are unavailable, sensor or capillary glucose values will be used to determine if the patient requires a treatment.

The following guidelines will be followed for the treatment of hypoglycemia.

- If glucose levels are < 3.3 mmol/L and associated with symptoms, the patient will self-administer 16g of oral carbohydrates to treat hypoglycemia. Glucose levels will be measured again in 15 minutes and the patient will self-administer another 16g of carbohydrates if glucose level is not > 3.3 mmol/L.
- If glucose levels are < 3.0 mmol/L irrespective of symptoms, the patient will self-administer 16g of oral carbohydrates to treat hypoglycemia. Glucose levels will be measured again in 15 minutes and the patient will self-administer another 16g of carbohydrates if glucose level is not > 3.3 mmol/L.

6.4.2 Treatment of Hyperglycemia

By default, treatment of hyperglycemia will be based on plasma glucose levels. However, in the event that plasma glucose measurements are unavailable, sensor glucose or capillary glucose values will be used to determine if patient requires a treatment.

If glucose level >18.0 mmol/L for two consecutive samples or if capillary glucose at the start/end of the study is >18 mmol/L, blood ketones will be checked.

- if ketones < 1.5 mmol/L (negative) and glucose level is maintained > 18.0 mmol/L over the next 2 hours:
 - The patient will be asked to change their infusion set.
 - Blood ketones will be re-assessed at the 2-hour mark (± 10 minutes). The hyperglycemia procedures will be repeated based on the results.
- if ketones < 1.5 mmol/L (negative) and glucose level falls below 18.0 mmol/L over the next 2 hours, no additional measures will be taken.
- if ketones < 1.5 mmol/L (negative) at the end of the study, the patient will be reminded by the research staff to take a correction bolus as per their standard practice.
- if ketones > 1.5 mmol/L (positive):
 - The study will be stopped, however the patient will not be discharged until acceptable blood ketone levels (lower than 1.5 mmol/L) are reached.
 - Additional insulin of 0.1 units/kg will be administered via a syringe by the research nurse. This step will be repeated every 2 hours (± 10 minutes) until blood ketones are corrected.

6.4.3 Stopping Criterion

The study will be stopped at the discretion of the investigators in the following circumstances:

- If no glucose measurements (plasma, capillary, or sensor glucose) are obtained for more than 2 hours during the 24-hour intervention visit.

7 Statistical Analysis

7.1 Study Endpoints

7.1.1 Primary comparisons

Percentage of time of glucose levels spent in target range (defined to be between 3.9 and 10.0 mmol/L). The following comparisons will be done:

- i) Fast-acting insulin-plus-pramlintide closed-loop delivery vs. fast-acting insulin-alone closed-loop delivery;
- ii) Regular insulin-plus-pramlintide closed-loop delivery vs. fast-acting insulin-alone closed-loop delivery.

7.1.2 Secondary endpoints and comparisons

1. Percentage of time of glucose levels spent in target range, comparing fast-acting insulin-plus-pramlintide closed-loop delivery vs. regular insulin-plus-pramlintide closed-loop delivery.
2. Percentage of time (8:00-8:00) of glucose levels spent:
 - a.* between 3.9 and 7.8 mmol/L; *b.* between 3.9 and 10 mmol/L; *c.* below 3.9 mmol/L; *d.* below 3.3 mmol/L; *e.* below 2.8 mmol/L; *f.* above 7.8 mmol/L; *g.* above 10 mmol/L; *h.* above 13.9; *i.* above 16.7 mmol/L.
3. Percentage of overnight time (23:00-8:00) of glucose levels:
 - a.* between 3.9 and 7.8 mmol/L; *b.* between 3.9 and 10 mmol/L; *c.* below 3.9 mmol/L; *d.* below 3.3 mmol/L; *e.* below 2.8 mmol/L; *f.* above 7.8 mmol/L; *g.* above 10 mmol/L; *h.* above 13.9; *i.* above 16.7 mmol/L.
4. Standard deviation of glucose levels as a measure of glucose variability.
5. Total insulin delivery.
6. Total pramlintide delivery.
7. Mean glucose level during: *a.* the overall study period; *b.* overnight period.
8. Mean insulin concentration.
9. Mean glucagon concentration.
10. Mean amylin concentration.
11. Number of participants experiencing hypoglycemia requiring oral treatment during: *a.* the overall study period; *b.* the night.
12. Gastrointestinal symptoms during the treatment optimization (i.e., the minimum 10 days prior to the 24-hour closed-loop visits) and during the 24-hour closed-loop visits.

7.2 Sample Size and Power Calculations

The power calculations aim to compare the effects of the interventions on the primary endpoint (time in target range), made at the $\alpha=0.05/2$ significance level: **i)** fast-acting insulin-alone closed-loop delivery and fast-acting insulin-plus-pramlintide closed-loop delivery; **ii)** fast-acting insulin-alone closed-loop delivery and regular insulin-plus-pramlintide closed-loop delivery.

The anticipated difference in time in target range was estimated from Weinzimer et al [6]. In that study, pramlintide increased the time spent in target range by 4%. In our study, we infuse pramlintide during the night too, and we anticipate that will improve glucose control even more. We anticipate that the insulin-plus-pramlintide closed-loop delivery will increase the percentage of time spent in target range by 7% (SD=10%) compared to the insulin alone closed-loop system. The standard deviation of the differences in the percentage of time in target range is estimated from our previous studies. It has always been between 9% and 11% for all comparisons that we usually make (sensor-augmented pump therapy, single-hormone artificial pancreas, and dual-hormone artificial pancreas).

Using the typical sample size formula for paired t-test, we calculated that 22 participants would provide 80% power at the 2.5% (0.05/2) significance level to detect differences between the interventions in the primary comparison. To account for anticipated drop-outs, technical problems, and remaining uncertainty in power calculations, 28 participants will be recruited.

Participants who do not complete one fast-acting insulin-alone closed-loop intervention and at least one insulin plus-pramlintide closed-loop intervention will be replaced in the enrollment process, will not be included in the analysis, and will not count toward the recruitment goal.

7.3 Level of Significance

5% significance threshold will be used to declare statistical significance. Bonferroni correction will be used for the two primary comparisons. No formal correction will be applied for the secondary comparisons.

7.4 Statistical Tests

The effect of the treatments on the continuous endpoints will be estimated using a linear mixed effect model. The hypothesis of no sequence effect will always be tested to check for carry-over effects. The model will be suited for repeated observations, i.e., adjusts for patient-level intracorrelation. Residual values from the regression model will be examined for an approximate normal distribution. If values are highly skewed, a transformation or nonparametric analyses will be used. Number of participants

requiring treatment for hypoglycemia will be compared between the two interventions using McNamer test.

8 Investigational Products

8.1 Pramlintide

Amylin, in the postprandial period, contributes to regulating glucose levels by delaying gastric emptying, suppressing nutrient-stimulated glucagon secretion, and increasing satiety. Pramlintide is a synthetic analog of the hormone amylin.

8.1.1 Administration

Pramlintide will be administered via a Medtronic pump (Minimed® Veo™ or 630G, Medtronic) at a fixed ratio of pramlintide per unit (U) of insulin. During the study, pramlintide will be infused at:

- 3 µg of pramlintide per unit (U) of insulin in the first 0-4 days of the optimization period
- 6 µg of pramlintide per unit (U) of insulin for the remaining of the optimization period and during the intervention visits.

8.1.2 Potential Risks

Pramlintide can cause the following side effects [9]:

- Severe low blood glucose level
- Nausea
- Vomiting
- Stomach pain
- Decreased appetite
- Headache
- Injection site reactions, including bruising, swelling or itching at the injection site.

8.1.3 Potential Benefits

Pramlintide has a potential to increase time in the target glucose range when co-administered with insulin.

8.1.4 Pregnancy

The effects of pramlintide on an unborn baby have not been identified [9].

9 Ethical and Legal Consideration

9.1 Good Clinical Practice

This study is to be conducted according to globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice, 1 May 1996), in agreement with the Declaration of Helsinki and in keeping with local regulations.

9.2 Delegation of Investigators Duties

The investigator should ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions. The investigator should maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

9.3 Participant Information and Informed Consent

After reading the relevant documents, the participant must give consent in writing. This consent must be confirmed by the personally dated signature of the participant and by the personally dated signature of the person conducting the informed consent discussions. A copy of the signed consent documents must be given to the participant. The original signed consent documents will be retained by the investigator. The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

It is suggested that the investigator inform the participant's primary physician about the participant's participation in the trial, if the participant has a primary physician other than the study investigator.

9.4 Confidentiality

Participant names will be kept in strictest confidence. Participants will be identified by their participant identification numbers which does not contain date of birth or initials. Study data stored on a computer will be stored in accordance with local data protection laws. The investigator will maintain a personal participant identification list (participant numbers with the corresponding participant names) to enable records to be identified. Participants' identifiers and contact details will be stored locally under strict security.

9.5 Approval of the Clinical Study Protocol and Amendments

Before the start of the study, the clinical study protocol, informed consent document, and any other appropriate documents will be submitted to the IEC/IRB with a cover letter or a form listing the documents submitted, their dates of issue, and the site for which approval is sought.

Before the first participant is enrolled in the study, formal written approval from the IEC/IRB must be obtained and all ethical and legal requirements must be met.

The IEC/IRB must be informed of all subsequent protocol amendments and administrative changes, in accordance with local legal requirements.

The investigator must keep a record of all communication with the IEC/IRB.

9.6 Record Retention

All study records must be kept according to ICH guidelines (details are provided in the Manual of Operations).

10 Adverse events

10.1 Adverse Events (AE)

An adverse event is any untoward medical occurrence or unfavorable and unintended sign in a subject administered a pharmaceutical product, biologic (at any dose), or medical device, whether or not considered related to the use of that product. This includes the onset of new illness and the exacerbation of pre-existing conditions.

Additionally, any event that is associated with or observed in conjunction with a product overdose (whether accidental or intentional) or a product abuse and/or withdrawal is also considered an adverse event. Adverse event is considered also malfunction or lack of function of any software or hardware part of the artificial pancreas system which results in symptoms.

The onset and end dates, severity and relationship (causality) to study drug/device/procedure will be recorded for each adverse event. Any action (treatment) or outcome (e.g., hospitalization, discontinuation of therapy, etc.) will also be recorded for each AE.

Adverse events will be collected from the time of signing the informed consent until the subject finishes his/her participation in the study, including non-interventional days.

After the last per-protocol contact the investigator does not need to actively monitor patients for AEs. However, if the investigator becomes aware of SAEs that occurred after the last per protocol contact and if there is suspected causal relationship to the study intervention, the SAEs will be reported by the investigator.

10.2 Classification of specific adverse events

Following are the specific diabetes related symptoms which will be reported as adverse events:

- Hypoglycemia: Hypoglycemic events will be recorded as an adverse event only if they reach level of “severe hypoglycemia”. Severe hypoglycemia is defined as the event required assistance of another person due to altered consciousness to actively administer carbohydrate, glucagon, or other resuscitative actions i.e., the participant was impaired cognitively to the point that he/she was unable to treat his/herself, was unable to verbalize his or her needs, was incoherent, disoriented, and/or combative, or experienced seizure or coma.

10.3 Severity (intensity) and causality of adverse events

Adverse events will be classified as mild, moderate or severe in severity (intensity) as follows:

- Mild: Discomfort noticed but no disruption of normal daily activity.
- Moderate: Discomfort sufficient to reduce or affect normal daily activity.
- Severe: Incapacitating with inability to work or perform normal daily activity

Adverse events will be at the same time classified assessed for causality to the study intervention. Medical judgement should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

- Yes: There is a reasonable causal relationship between the investigational product administered and the AE.
- No: There is no reasonable causal relationship between the investigational product administered and the AE.

10.4 Reporting Serious Adverse Events (SAE)

A serious adverse event (SAE) is defined as any AE which:

- results in death

- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly/birth defect

It is the responsibility of the investigator to notify their local ethics committee of all reportable SAEs following local REB guidelines.

SAE which are unexpected and causal relation to study drug (pramlintide) is suspected will be reported to Health Canada in a following manner:

- a. Where it is neither fatal nor life-threatening, within 15 days after becoming aware of the information;
- b. Where it is fatal or life-threatening, within 7 days after becoming aware of the information. Within 8 days after having initially informed Health Canada of the fatal or life-threatening ADR, submit as complete a report as possible. Follow-up reports of fatal or life-threatening reactions **must** include an assessment of the importance and implication of the findings, including relevant previous experience with the same or similar drugs.

All (S)AEs, including those persisting after trial completion must be followed up until they have resolved, have been sufficiently characterized, or no further information can be obtained.

10.5 Pregnancy

If subject becomes pregnant during the study, her participation in the study will be discontinued. The outcome of the pregnancy must be followed up and reported.

11 References

1. Levetan, C., et al., *Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglyceride excursions among patients with type 1 diabetes intensively treated with insulin pumps*. *Diabetes Care*, 2003. **26**(1): p. 1-8.
2. Ratner, R.E., et al., *Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in Type 1 diabetes mellitus: a 1-year, randomized controlled trial*. *Diabet Med*, 2004. **21**: p. 1204-1212.
3. Huffman, D.M., G.W. McLean, and M.A. Seagrove, *Continuous subcutaneous pramlintide infusion therapy in patients with type 1 diabetes: observations from a pilot study*. *Endocr Pract*, 2009. **15**(7): p. 689-95.
4. Heptulla, R.A., et al., *Twenty-four-hour simultaneous subcutaneous Basal-bolus administration of insulin and amylin in adolescents with type 1 diabetes decreases postprandial hyperglycemia*. *J Clin Endocrinol Metab*, 2009. **94**(5): p. 1608-11.
5. Hovorka, R., *Closed-loop insulin delivery: from bench to clinical practice*. *Nat Rev Endocrinol*, 2011. **7**(7): p. 385-95.
6. Weinzimer, S.A., et al., *Effect of Pramlintide on Prandial Glycemic Excursions During Closed-Loop Control in Adolescents and Young Adults With Type 1 Diabetes*. *Diabetes Care*, 2012. **35**: p. 1994-1999.
7. Sherr, J.L., et al., *Mitigating Meal-Related Glycemic Excursions in an Insulin-Sparing Manner During Closed-Loop Insulin Delivery: The Beneficial Effects of Adjunctive Pramlintide and Liraglutide*. *Diabetes Care*, 2016. **39**(7): p. 1127-34.
8. Weyer, C., et al., *Pramlintide Reduces Postprandial Glucose Excursions when added to Regular Insulin or Insulin Lispro in Subjects with Type 1 Diabetes*. *Diabetes Care*, 2003. **26**(11): p. 3074-3079.
9. AstraZeneca, *Symlin (pramlintide acetate) Product Monograph*. 2015.
10. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study, G., *Prolonged nocturnal hypoglycemia is common during 12 months of continuous glucose monitoring in children and adults with type 1 diabetes*. *Diabetes Care*, 2010. **33**(5): p. 1004-8.
11. Choudhary, P., et al., *Frequency of biochemical hypoglycaemia in adults with Type 1 diabetes with and without impaired awareness of hypoglycaemia: no identifiable differences using continuous glucose monitoring*. *Diabet Med*, 2010. **27**(6): p. 666-672.
12. Chico, A., et al., *The continuous glucose monitoring system is useful for detecting unrecognized hypoglycemia in patients with type 1 and type 2 diabetes but is not better than frequent capillary glucose measurements for improving metabolic control*. *Diabetes Care*, 2003. **26**(4): p. 1153-1157.
13. Cryer, P.E., *Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes*. *Diabetologia*, 2002. **45**(7): p. 937-48.