Study Protocol

Title: Glucagon Response to Prandial Insulin Administration in Persons with Type 1 Diabetes

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Summary of Changes	
Version 2.0 to 2.1	Removing Dr. Zelada Castro as Primary Investigator and adding Dr. Janet McGill
Version 1.0 to 2.0	Adding an additional blood sample for future research
	Changes to blood draw volumes
	Clarification of location data & sample storage
	Clarification of location of lab for processing samples

Glucagon Response to Prandial Insulin Administration in Persons with Type 1 Diabetes

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OVERVIEW

Glucagon regulation and response in persons with T1D at the basal state and in response to various stimuli remains unclear. Dr. Philip Cryer has previously reported that, in T1D young adults with a course of the disease of 16+9 years, the absence of endogenous insulin secretion results in increased glucagon secretion after a mixed meal, concluding that endogenous insulin reciprocally regulates the alpha-cell glucagon secretion [1,2] and also suggesting that glucagon dysregulation may play an important role in post-prandial hyperglycemia in T1D. Interestingly, recent research on human islets have shown that insulin inhibits counter-regulatory glucagon secretion by a paracrine effect mediated by SGLT2-dependent stimulation of somatostatin release [3].

T1D course: [4]

In general, loss of 80% of beta cell function is needed to develop severe hyperglycemia. Endogenous insulin secretion is almost entirely lost within 3 to 5 years after the diagnosis [5]. In children with new diagnosis of T1D, glucagon levels were highly associated with post prandial blood glucose, but not with HbA1c level [6]. It has also been reported that postprandial hyperglucagonemia worsens significantly while C-peptide secretion declines during the first year of the disease [7]. Also, in children with greater than five years of T1D, postprandial glucagon levels increased 160% from one to sixty months after diagnosis [8] and in those with disease duration of 6.9 + 4 years, glucagon levels were strongly correlated with post-prandial glucose and mildly correlated with HbA1C levels [9]. A study in T1D adults performed in China showed that post-prandial glucagon levels were higher in T1D patients in the first year of the disease compared to those with longer course of the disease, and that hypoglycemic events were lower in newly diagnosed patients [10], suggesting that glucagon secretory function may become impaired with longer duration of the disease. These studies suggest that glucagon dysregulation could impact glucose control with exogenous insulin, and that this dysregulation may change with disease duration.

Insulin Timing and Hyperglycemia in T1D:

Post-prandial hyperglycemia adds to glucose variability, difficulty with insulin dosing and overall instability of glucose control. A recent analysis from the T1D Exchange study, that included 4768 participants younger than 26 years with a clinical diagnosis of T1D for at least 1 year showed that 21% of the participants reported administering insulin several minutes before, 44% immediately before, 10% during, and 24% after meal. Interestingly, participants who reported administering insulin during or after a meal were more likely to report missing ≥1 mealtime insulin dose per week compared with those who administered insulin before meals ([11]). In addition, a sub-analysis showed that participants who dose mealtime insulin in the postprandial period have poorer glycemic control and greater frequency of severe hypoglycemia ([12]). However, despite these recent results, patients are often advised to give insulin after eating

because caloric intake is uncertain.

Glucagon Regulation:
Different models have been used to study glucagon regulation [13,14,15,16]. Since both hyperglycemia and hypoglycemia stimulate alpha cells to produce glucagon, both the lack of intra-islet insulin and this U-shaped glucagon response contribute to glucose dysregulation in T1D [17,18]. It has been reported that in T1D patients with a duration of the disease of 26.4+7.5 years, glucagon levels increased in response to the OGTT under euglycemic and hyperglycemic conditions [19], however the relative contribution of hyperglucagonemia to post-prandial glucose rise in the presence or absence of pre-prandial insulin has not been clearly elucidated. Whether appropriately timed exogenous insulin can modify the glucagon response to glucose fluctuations has not been studied.

Research Objective:

To characterize the glucagon response to meal-time hyperglycemia and to compare the difference in glucagon secretion when mealtime bolus insulin is given before the meal versus after the meal with the objective of understanding factors that contribute to the peak post-prandial blood glucose and AUC of blood glucose after a mixed meal in this target population.

Research Hypothesis:

1. Patients with higher glucagon responses to a mixed meal stimulus will have higher peak post-prandial glucoses and greater AUC of post-prandial glucose.
2. Bolus dose insulin given pre-meal (20 min before meal) will result in lower peak post-prandial glucose, lower AUC and lower post-prandial glucagon levels
3. Bolus dose insulin given post-meal (20 min after meal) will result in higher peak post-prandial glucoses, higher AUC of post-meal glucose and higher glucagon levels
4. There will be a correlation between peak post-prandial glucagon response and post-meal glucose AUC, 0 to 180 minutes

Potential Contribution:

Glucagon regulation and response in persons with T1D at the basal state and in response to various stimuli remains unclear. An important gap in our knowledge is whether the timing of prandial insulin doses affects the glucagon response to a hyperglycemic stimulus in patients with T1D who have undetectable C-peptide. Understanding the mechanisms behind the higher post-prandial peak in glucose with delayed insulin administration may help clinicians guide appropriate timing of insulin administration for T1D patients with long course of the disease

METHODS

Recruitment:

Subjects will be recruited from the investigators' private patient population. Screening will be done over the phone and with review of medical records.

Inclusion criteria:

- Persons of all races, ethnicity and genders will be included, Age ≥18
- Type 1 Diabetes for >5 years
- HbA1c ≤9.5%
- Using either MDI or insulin pumps
- Patients using CGM may continue the use during the study, however glucoses will be measured by laboratory methods.
- Hemoglobin: Female 10.8 -17.05 g/dL and Males 11.7-19.25 g/dl
- Hematocrit: Female 32.8-48.7% and Males 37-55.3%

Exclusion criteria:

- Type 2 diabetes, monogenic diabetes, pancreatic diseases
- eGFR <60 ml/min/1.73m2.
- Use of daily steroids, any route, for any purpose
- Pregnancy, prisoners, other vulnerable populations or persons unable to understand the protocol and provide written informed consent

Data Collection:
See Appendix A

LOCATION OF STUDY VISITS
The study will be conducted in the Washington University-Diabetes Center, Center for Advanced Medicine 13B and/or the Intensive Research Unit, Center for Outpatient Health 5th floor.

PROCEDURES

Study Visits (Visits will take approximately 4 hours)

- Informed Consent may be performed on a visit prior to Visits A/B – subjects will be provided informed consent
- Visits A and B will be performed in random order, these visits will take place on 2 separate days within 1 week of the first visit
- Demographics, Obtain medical and medication history, including review of diabetes history, medications and allergies will be recorded at the first study visit. Insulin doses and effectiveness of current carbohydrate ratio will be evaluated and recorded. Mealtime insulin doses will be established at the first study visit.
- Baseline glucose should be 80 to 180 mg/dl. In case baseline glucose is not in the target range, the investigator will determine if the subject should return and the study visit rescheduled.
- Urine pregnancy test for women of childbearing potential
- Brief physical exam performed at first study visit
- Vital signs, height and weight performed at each visit
- Meal tolerance tests with timed samples collected as noted below performed at Visit A & B. Administration of liquid meal and Mealtime insulin to be given via injection by a study nurse to standardize the administration procedure.
- C-peptide may be checked with the fasting labs at the first study visit
- A sample for future research will be obtained, about 5ml (1tsp.)
- Whole blood will be collected in vacutainers via an indwelling peripheral venous catheter with nursing supervision from the diabetes center at Washington University. Maximum blood collection will be about 20ml (4 tsp).

Visit A

- Glucose levels done fasting, 30, 60, 120, and 180 minutes
- Glucagon levels done fasting, 30, 60, 120, and 180 minutes
- Usual insulin dose will be administered 20 minutes prior to a mixed meal challenge with Ensure Plus*

Visit B

- Glucose done fasting, 30, 60, 120, and 180 minutes
- Glucagon levels done fasting, 30, 60, 120, and 180 minutes
- Usual insulin dose will be administered 20 minutes after the mixed meal challenge with Ensure Plus*

*Ensure Plus (Abbott Nutrition) at 6cc/kg (max 360 cc), content per 100 ml: carbohydrate 21.5 grams, protein 5.5 grams, with nursing supervision.

PROTECTION OF CONFIDENTIALITY

Data Security

Study data may be stored in REDCAP or on WUSM protected servers as well as collective data which will be stored in a locked cabinet in a locked office. Study staff will be required to have their own logon/password to access electronic data and access to paper charts will be limited to authorized study staff.

Blood Samples may be processed and stored in the Diabetes Center or in the Center for Outpatient Health clinical research unit (CRU) in a locked room with access restricted to study staff or delivered directly to the Washington University Core lab and the investigators lab for handling and analysis. Blood samples will be coded before sending to the laboratory. The samples may include a subject identification number and date of birth.

Plasma and serum specimens will be stored in a Washington University School of Medicine research Laboratory for future studies of other metabolic parameters relating to pancreatic function and/or insulin signaling; 5 ml of plasma and serum will be collected at one of the visits.

ASSESSMENT OF RISKS AND BENEFITS

Potential risks include:

- Mild pain from IV catheter insertion
- Hypoglycemia after insulin administration. Patient will be monitored by a physician and a nurse during the whole visit.

Methods

Alpha cell function will be assessed by measuring fasting and post-prandial glucagon and calculation Area under the Curve ratio (AUC-Glucagon 0-180min/AUC-Glucose 0-180min). Some of the participants characteristics will include in the study are: Demographics, BMI, history of DKA, DKA at onset of diagnosis, diabetes comorbidities (Retinopathy, cardiovascular, nephropathy, neuropathy), concomitant autoimmune disease, type and dose of insulin,

Methods of Data Analysis

Data analysis will be performed using STATA 15.1 for Windows (StataCorp LP, College Station, Texas, United States). The sample size calculation assumes that the peak postprandial glucose will increase by 80 +/-20 mg/dl in the pre-meal insulin group and 140 +/-20 mg/dl in the post-meal group. So, the expected mean peak of postprandial glucose will be up to 220 mg/dL for Visit A and mean peak postprandial glucose will be up to 280 mg/dL for Visit B. The sample size will have 80% power to detect a difference between groups at $p<0.05$. We have assumed correlation between visits A and B of 0.5. The sample size was corrected by an anticipated loss of follow up of 10% of the participants. The calculated sample size is 10 participants with full

data sets available for 8 participants. The study randomization is designed as follows: 5 participants will first attend Visit A, and then Visit B. The 5 remaining participants will first attend Visit B and then Visit A. AUC of post-prandial glucose and glucagon and maximum postprandial peak of glucose and glucagon will be analyzed by using paired T student. Relationships between variables were assessed by a Spearman's correlation test.

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Date:

Patient Name:

DOB:

MRN:

Evaluator:

Visit A:

BP: HR: RR: T: Weight: BMI:

1. Demographic information:
    a. Age
    b. Sex:
    c. Married:
    d. Time of the disease (T1D)
    e. History of DKA
    f. DKA at onset of diagnosis
    g. Diabetes comorbidities (Retinopathy, cardiovascular, nephropathy, neuropathy)
    h. Concomitant autoimmune disease
    i. History of hypoglycemia,
    j. Hypoglycemia unawareness
2. T1D:
    - Diabetes medications:
        a. Oral:
        b. Non-injectable insulin
        c. Insulin
    - ICR
    - Statins:
3. Allergies:

4. Meal time prandial insulin dose to be used (in second visit)
5. Physical Examination:
    a. General:
    b. HEENT:
    c. Cardio:
    d. Lungs:
    e. GI:
    f. Extremities:
    g. Skin:
    h. Neuro:
6. Labs:
    - Fasting C-peptide
    - CBC (if required)
    - GFR (if required)
    - A1C (if required)
7. Glucose Target prior intervention: (Baseline glucose should be 80 to 140 mg/dl. In case baseline glucose is not in the target range, the study visit will be rescheduled): Yes, No
8. Intervention:
   Usual insulin dose will be administered 20 minutes **prior** to a mixed meal challenge with Ensure Plus
    - Glucose levels:
        - Fasting:
        - 30 min:
        - 60 min:
        - 120 mins:
        - 180 min:
    - Glucagon levels:
        - Fasting:
        - 30 min:
        - 60 min:
        - 120 mins:
        - 180 min:
9. Was the intervention hold? Yes, No Reason:

Visit B:

BP: HR: RR: T: Weight: BMI:

1. Glucose Target prior intervention: (Baseline glucose should be 80 to 140 mg/dl. In case baseline glucose is not in the target range, the study visit will be rescheduled): Yes, No
2. Physical Examination:
    a. General:
    b. HEENT:
    c. Cardio:
    d. Lungs:
    e. GI:
    f. Extremities:
    g. Skin:
    h. Neuro:

3. Intervention

Usual insulin dose will be administered 20 minutes **post** to a mixed meal challenge with Ensure Plus

- Glucose levels:
    - Fasting:
    - 30 min:
    - 60 min:
    - 120 mins:
    - 180 min:

- Glucagon levels:
    - Fasting:
    - 30 min:
    - 60 min:
    - 120 mins:
    - 180 min:

4. Was the intervention hold? Yes, No Reason: