Evaluating the Benefits of Physiologic Insulin Delivery

Study Protocol and Statistical Analysis Plan

NCT04416737

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SPECIFIC AIMS

As of 2015, 30 million Americans (9.4% of the US population) had diabetes and it was the seventh leading cause of death based on 79,535 death certificate [131]. In addition to the American Diabetes Association (ADA) reported 1.25 million people in the United States with type 1 diabetes, about 29% of the 29 million individuals with type 2 diabetes use insulin [132]. Long-term complications of hyperglycemia include retinopathy, nephropathy, neuropathy, and cardiovascular disease [133, 134]. The latest position statement by the ADA recommends a hemoglobin A1c goal of <7% in adults and <7.5% in pediatric age-groups [55, 135]. This goal challenges even the most motivated, knowledgeable and socioeconomically privileged patient. Despite a $327 billion investment in diabetes healthcare in 2017, nearly half of those with diabetes have a hemoglobin A1c above goal [136] and 31% have a hemoglobin A1c >9% [131].

Insulin replacement treatment is a significant burden with a narrow therapeutic margin, too much insulin results in acutely debilitating hypoglycemia and too little causes hyperglycemia that, over years, results in microvascular and coronary artery disease. In 2016, following years of clinical trials performed at Stanford and other centers, the FDA approved the Medtronic 670G "hybrid" closed-loop system which automatically controls insulin delivery based on continuous glucose monitor (CGM) data [137]. Unfortunately, current subcutaneous insulin pharmacokinetics are too slow to provide meal coverage without announcement and the kinetics make it necessary to choose between hypoglycemia and hyperglycemia prevention, inevitably generating alarms when the user hits the opposite extreme [138]. Additionally, there are many who stop using CGM or insulin pumps; there reasons for discontinuation include: alarms, trust in the technology and dislike of wearing devices [102]. An implanted "invisible" pump could deliver intraperitoneal insulin with more rapid pharmacokinetics providing full closed-loop glucose control and thereby reduce the burden and morbidity of diabetes.

Prior investigation of intravenous and intraperitoneal automated insulin delivery technology were limited by the absence of practical, accurate CGM technology. Consequently, these approaches were erroneously discarded. CGM, catheter and insulin pump technology continue to evolve rapidly, but the optimal location of insulin delivery for full closed-loop control remains unknown. *We posit that long-term intraperitoneal insulin delivery will be more physiologic with multiple benefits including improved body composition and BMI (important additional cardiovascular risk factors), bone health and user experience while at the same time providing the necessary kinetics and glucagon response for fully automated insulin delivery.* Intraperitoneal insulin delivery will be simulated from available data with assessment of multiple control strategies.

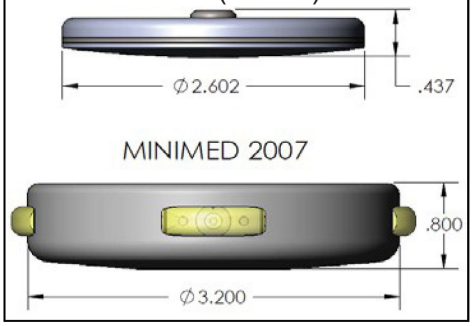
AIM 1: To identify unique benefits of long-term intraperitoneal insulin use. Utilizing data being collected from Dr. Eric Renard's (co-mentor) cohort of intraperitoneal users with type 1 diabetes, we will evaluate body composition, bone health and human factors compared to matched controls using subcutaneous insulin.
Hypothesis 1: Long-term intraperitoneal use is associated with decreased BMI, body fat percentage and visceral adiposity with improved markers of bone health, and more positive opinions of diabetes technology.

AIM 2: To describe the pharmacokinetics and compare glucagon response to hypoglycemia of novel insulin formulations delivered via upper peritoneal, lower peritoneal and subcutaneous routes.
Hypothesis 2: Pharmacokinetics of intraperitoneal insulin will be faster and lead to greater counter-regulatory glucagon response than subcutaneous insulin. Upper peritoneal delivery will be faster than lower peritoneum.

AIM 3: To use available intraperitoneal pharmacokinetic and counter-regulatory data to perform *in silico* modeling and test performance with various control strategies. The optimal algorithm will be the least computationally demanding that provides glycemic control in range (70-180mg/dL) over a variety of physiologic conditions.
Hypothesis 3: With more rapid pharmacokinetics we can utilize more straightforward control strategies to achieve >90% time in range.

This work will deliver a greatly needed control strategy for intraperitoneal insulin delivery that allows for full closed-loop glucose control. Our results will help guide the development of an implanted insulin pump being developed by PhysioLogic Devices (**Figure 1**). An efficient control algorithm will be needed to run on an embedded microcontroller with limited power. The goal of our combined efforts will be multi-center human clinical trials to achieve full closed-loop, reducing the burden of diabetes, and achieving optimum glycemic control.

Figure 1. ThinPump vs Prior MiniMed 2007 (inches)

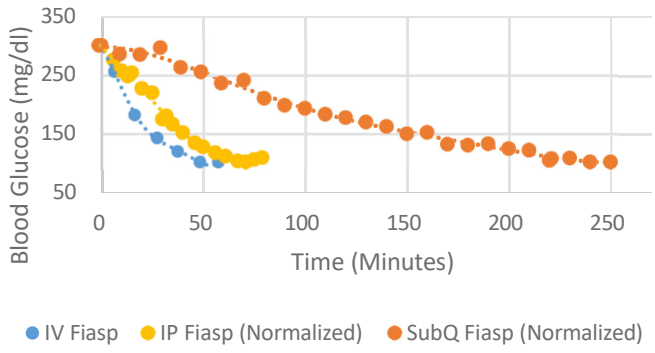


AIM 2: To describe the pharmacokinetics and compare glucagon response to hypoglycemia of novel insulin formulations delivered via upper peritoneal, lower peritoneal and subcutaneous routes.
Hypothesis 2: Pharmacokinetics of intraperitoneal insulin will be faster and lead to greater counter-regulatory glucagon response than subcutaneous insulin. Upper peritoneal delivery will be faster than lower peritoneum.

Rationale/Preliminary Data: All prior intraperitoneal pharmacokinetic studies used only concentrated regular insulin [2, 149, 150], which may be too slow to provide full closed-loop insulin delivery without meal announcement [117, 119, 124]. Dr. Appel, an R01-funded Stanford collaborator, has developed excipients that stabilize insulin analogs [234] for implanted delivery. Novo Nordisk received FDA approval for fast-acting insulin aspart (Fiasp) in 2017. It is the fastest subcutaneous insulin and contains the excipients niacinamide and L-arginine hydrochloride thought to increase the formation of biologically active Aspart monomers, accelerate insulin absorption and add stability [235]. A description of intraperitoneal Fiasp kinetics, as well as counter-regulatory hormonal factors that may counter hypoglycemia is needed. Upper versus lower peritoneal delivery may also affect insulin kinetics [2]. Another important benefit of intraperitoneal insulin is restoration of glucagon response in longstanding diabetes, which has been studied in a small number of intraperitoneal users [21-25]. We conducted a preliminary proof-of-concept study on intravenous, intraperitoneal and subcutaneous Fiasp administration in one subject to compare kinetics in each compartment (**Figure 2**).

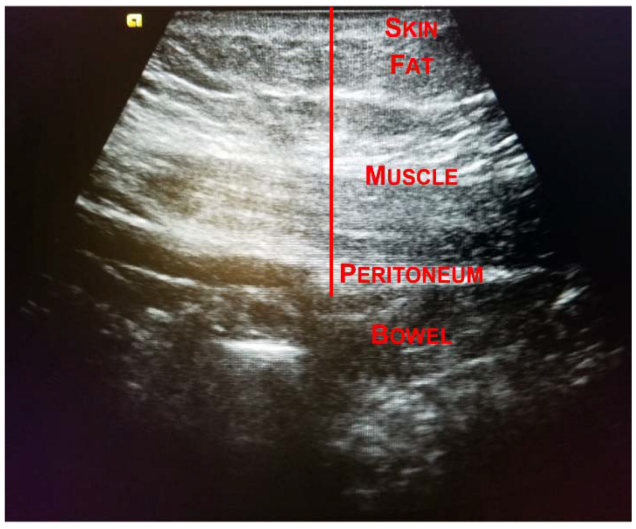
Glucose data was collected by raising blood sugar to ~300mg/dL for 30 minutes with only basal insulin running (no recent boluses) then administering a fixed dose of Fiasp in each compartment. The experiment concluded when glucose stabilized for 15 minutes. In each compartment Fiasp had a shorter duration of action than prior studies with regular insulin (IV: 50mins vs 90 mins [236], IP: 85mins vs 180mins [237] and SubQ: 4hrs vs 8.4hrs [238]). Intravenous and intraperitoneal insulin provide substantially more rapid glucose reduction than subcutaneous. Ultrasound-guided intraperitoneal insulin administration was painless.

Figure 2. Glucose Response to IV, IP and Subcutaneous Fiasp



Research Design: This will be a descriptive pharmacokinetic study of upper and lower versus subcutaneous Fiasp, with assessment of glucagon response to insulin-induced hypoglycemia. 15 healthy adult participants with type 1 diabetes on insulin pump and continuous glucose monitor will be consented and asked to come in for three visits separated by at least one week. Before any other invasive procedure the abdominal area will be assessed by an experienced interventional radiologist with gray-scale and color Doppler ultrasound to determine the safest puncture site (**Figure 3**). Gray-scale sonography will be used to find the area that provides the most separation between the anterior abdominal wall and the bowel loops. If the experienced interventional radiologist feels no safe area exists, the subject will be excluded.

Figure 3. Anterior Abdominal Ultrasound with Needle Placement (Red Line)

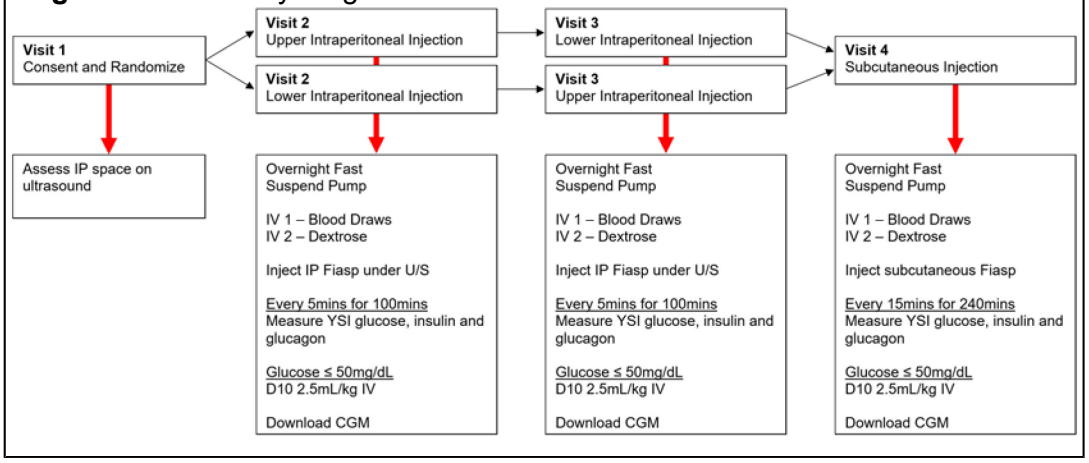


Participants will fast overnight and suspend subcutaneous insulin delivery approximately 1 hour prior to each procedure. For the first two visits, participants will be randomized to either upper or lower peritoneal injection. An IV will be inserted for blood draws for YSI glucose, insulin, and glucagon levels. Another IV will be placed for dextrose administration. Color Doppler sonography of the puncture site will be performed to visualize the epigastric vessels and verify they are at least 1 cm away from the determined puncture site. Under sonographic guidance, a 22-gauge needle is inserted at the selected site with the needle angled 45° caudally. Real-time

visualization of the needle insertion through the subcutaneous tissue, rectus abdominis muscle, and peritoneum will be performed. The peritoneum is seen as an echogenic line just deep in relation to the rectus abdominis muscle fibers. The needle will be taken to the edge of this echogenic line which may be associated with minimal momentary discomfort [239]. Approximately 0.25 units/kg Fiasp will be administered through the needle and the needle removed. Following injection, blood collection and measures will occur every 5 minutes for either 150 minutes or until glucose concentration has a stable trend. A 10% dextrose bolus of 2.5mL/kg will be provided following a blood sugar reading ≤50mg/dL. After 150 minutes have elapsed or glucose remains stable within 10mg/dL for 2 time points, the patient will resume their home insulin regimen. Data from the patient's CGM will be downloaded.

At least one week from the last peritoneal injection a similar subcutaneous procedure will take place. An IV will be inserted for blood draws for YSI glucose, insulin, and glucagon levels. Another IV will be placed for dextrose administration. Approximately 0.2 units/kg Fiasp will be administered by subcutaneous injection. Following injection, blood collection and measures will occur every 15 minutes for 6 hours or until glucose is stable. A 10% dextrose bolus of 2.5mL/kg will be provided following a blood sugar reading ≤50mg/dL. After 6 hours have elapsed or glucose remains stable within 10mg/dL for 2 time points, the patient will resume their home insulin regimen. Data from the patient's CGM will be downloaded. See **Figure 4** for study diagram.

Figure 4. SA2 Study Diagram



Statistical Considerations and Power Analysis: Using analyses appropriate for paired measures, we will employ paired t-tests or the analogous non-parametric test (e.g., Wilcoxon signed-rank) to assess differences in pharmacokinetic features between delivery mechanisms. In the prior 2000 study by Oskarsson and colleagues, 7 subjects with longstanding type 1 diabetes receiving subcutaneous and intraperitoneal insulin underwent induced hypoglycemia with IV insulin and had glucagon measured at baseline and during hypoglycemia [1]. The maximal glucagon response subtracted from baseline was 7.5 ± 3.0 pg/mL on subcutaneous insulin and 17.0 ± 3.1 pg/mL on intraperitoneal insulin. Assuming a type I error rate of 0.05, 15 individuals will provide >90% power to detect an effect size of 1.0 (maximal change in glucagon of 3.0).

Expected Outcomes: We will test if there is a significant difference in glucagon response during hypoglycemia between intraperitoneal and subcutaneous administration. A mean pharmacokinetic curve will be generated from the above data for upper/lower intraperitoneal and subcutaneous insulin administration. We anticipate that intraperitoneal Fiasp will have faster pharmacokinetics than subcutaneous delivery, and that intraperitoneal delivery will result in a more significant counter-regulatory glucagon response. The upper peritoneal pharmacokinetic profile may be more rapid than lower peritoneal delivery.

Potential Pitfalls & Alternative Strategies: There has been only 1 reported case of inadvertent bowel puncture with ultrasound guidance, occurring after the probe was removed and the needle unintentionally advanced. The patient received antibiotics and had no clinical sequelae [240]. We are deeply vested in the safety of our human subjects. All invasive procedures will be performed by an experienced interventional radiologist. To avoid this specific complication we will only take the needle tip to the edge of the visceral peritoneum, use a smaller needle and continue ultrasound recording until the needle is removed. The procedure to access the intraperitoneal space is derived from that used to place intraperitoneal catheters for peritoneal dialysis. An alternative procedure would be to perform the study in those with existing peritoneal catheters. Subjects with peritoneal dialysis catheters likely have impaired renal clearance or have recently received kidney transplant, which would alter pharmacokinetics. We have chosen not to use existing intraperitoneal pump users since prolonged suspension of insulin delivery may increase the risk of occlusions. We use Fiasp as it is the fastest subcutaneous insulin available. 1-hour suspension reduces plasma insulin with low risk of ketosis [241].

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Type 1 Diabetes Mellitus

2.2. Eligibility Criteria

Eligibility Criteria
To be eligible for the study, a subject must meet the following criteria:
1. 18-60 years of age
2. Clinical diagnosis of type 1 diabetes
3. On insulin pump therapy and continuous glucose monitor (CGM) for at least 3 months
4. Ability to safely receive intraperitoneal injection
5. For females, not currently known to be pregnant
6. Understanding and willingness to follow the protocol and sign informed consent
7. Ability to speak, read and write in the language of the investigators

Exclusion Criteria
The presence of any of the following is an exclusion for the study:
1. Diabetic ketoacidosis in the past 3 months
2. Severe hypoglycemia resulting in seizure or loss of consciousness within 3 months prior to enrollment
3. Pregnant or lactating
4. Active infection
5. A known medical condition that in the judgment of the investigator might interfere with the completion of the protocol
6. Known cardiovascular events in the last 6 months
7. Known seizure disorder
8. Inpatient psychiatric treatment in the past 6 months
9. Lack of stability on medication 1 month prior to enrollment including antihypertensive, thyroid, anti-depressant or lipid lowering medication.
10. Suspected drug or alcohol abuse
11. Chronic kidney disease (GFR < 60 mL/min/1.73mA2)

2.3. Age Limits Min Age: 18 Years Max Age: 60 Years

INCLUSION OF WOMEN, MINORITIES, AND CHILDREN

We will attempt to recruit an equal gender distribution and minority population representative of the surrounding demographic of people with type 1 diabetes. We are excluding children in these initial studies of intraperitoneal insulin injections. We hope to eventually be able perform studies on this more vulnerable population to improve modeling.

RECRUITMENT AND RETENTION PLAN

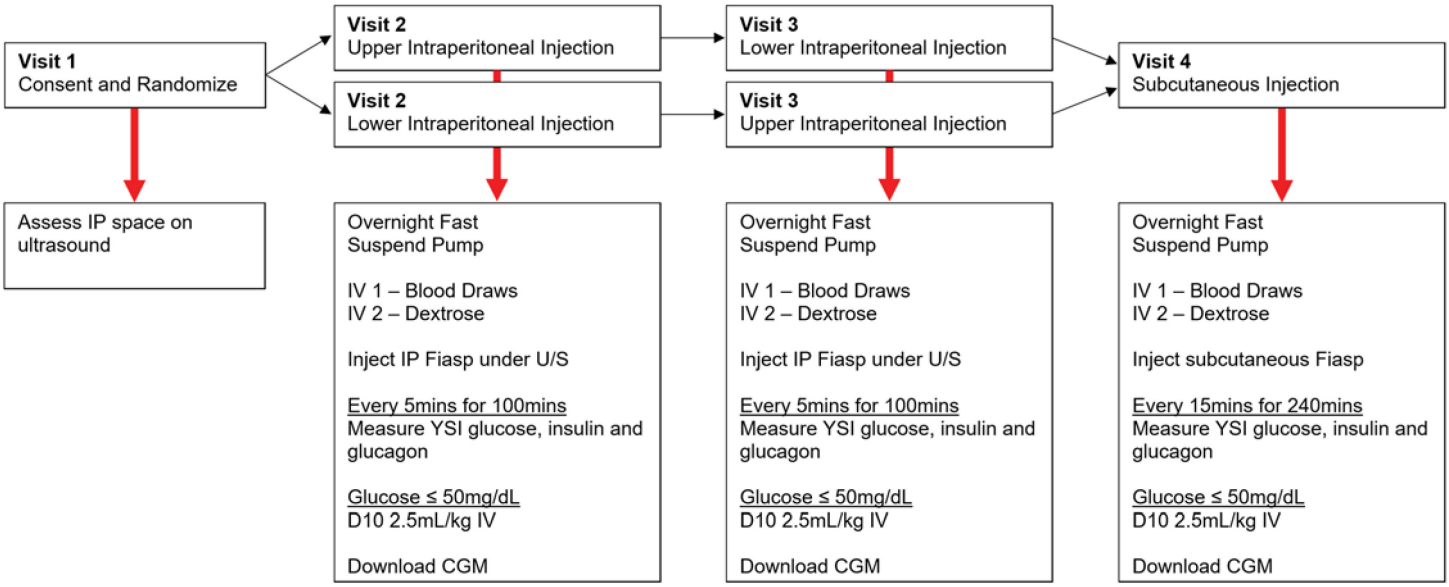
We will contact adult with type 1 diabetes using our existing database of patients who have consented to be contacted about future diabetes research studies. A short description of the present study will be given to them via e-mail, phone or in-person. We will also recruit subjects at the time of their scheduled appointments. A one-page brochure will be prepared and sent to providers who have referred patients to us in the past. Information on the study will also be posted on our website, and the information provided on the brochure will also be made available to diabetes support groups in our area and at local events hosted by JDRF and CarbDM. If a person expresses interest, then the study will be presented in detail. The study will be eligible to adults with type 1 diabetes, in good health, who are not currently enrolled in other intervention studies. Subjects participating in a registry study (no interventions) may participate.

Participants will be informed that the study will require 3 visits, at least 1 week apart. We will obtain and verify contact information. The intraperitoneal experiments will be conducted first, as this is the more invasive intervention. To reduce unnecessary exposure, we will only perform the subcutaneous intervention once we are certain that we can safely perform intraperitoneal injections. Patients completing the intraperitoneal visits are likely to return for subcutaneous testing since this represents standard of care for insulin delivery.

STUDY TIMELINE

We intend to perform specific aim 2 during the second and third years (1/2022-6/2023) of funding. See **Figure 1** for study diagram.

Figure 1. SA2 Study Diagram



We plan to recruit 15 subjects, and each individual will need to be consented and come in for three visits 1 week apart. Given the amount of testing and monitoring needed for each subject, we will perform testing on 1 subject at a time. Allocating 3 weeks for each participant gives a total study duration of around 1 year. See **Table 1** for study timeline.

Table 1. Study Timeline

Task	**Estimated Completion Date (Month / Year)**
Finalization of protocol, informed consent	1/2022
IRB Approval	2/2022
Major equipment, materials procured	6/2022
DSMB committee planning	6/2022
Study staff recruitment and training complete	6/2022
25% subjects complete study	9/2022
50% subjects complete study	12/2022
75% subjects complete study	3/2023
Final Report	6/2023

PROTECTION OF HUMAN SUBJECTS

1. Risks to Human Subjects

Human Subjects Involvement, Characteristics, and Design
In Aim 2 we will require human subjects to collect real-world pharmacokinetic data and compare glucagon response to hypoglycemia for insulin Fiasp delivered via upper peritoneal, lower peritoneal and subcutaneous routes. 15 healthy adult participants (age 18-60 years) with type 1 diabetes on insulin pump and continuous glucose monitor will be recruited. Patients with other chronic health conditions must demonstrate stability on current treatment regimens for at least 3 months. Subjects will be excluded if they have had recent severe hypoglycemia or diabetic ketoacidosis. Pregnant or breastfeeding individuals and those with medical conditions that alter insulin resistance, insulin clearance, mentation, seizure threshold or increase the risk of major adverse cardiac events will also be excluded.

The diabetes clinic at Stanford Hospital and Clinics serves a large, diverse population with over 400 adults with type 1 diabetes. This population represents a diverse mix of urban, suburban, and rural patients from a variety of racial and ethnic backgrounds.

Sources of Materials
Any individually identifiable private information about human subjects will be obtained at enrollment in a private room. Health information related to this study will include name, age, gender, race/ethnicity, diabetes duration, height/weight, pregnancy test results if female, date of study visits, insulin doses, hemoglobin A1c, continuous glucose monitoring results, measures of YSI glucose and insulin/glucagon ELISA. During the procedure, venous blood will be collected and appropriately disposed of when lab processing is complete. For security and confidentiality purposes, subjects will be assigned an identifier that will be used instead of their name. Study doctors and study coordinators are responsible for de-identifying the data. Dr. Lal and the research staff involved with this study will have access to the data and specimens. Staff will be current in HIPAA and CITI training, and will be reminded during training for this specific study to maintain privacy of participants and confidentiality of the data and specimens.

Potential Risks
Participation includes travel to Stanford and possible missed work that may pose a financial hardship. Injection and catheter placement carry common risks of discomfort, bleeding, bruising and infection. For more invasive intraperitoneal catheter insertion procedures (rather than injection utilizing our technique), there has been one reported case of bowel puncture, treated with antibiotics, without clinical sequelae. All procedures will be carried out by experienced providers, further decreasing the risk of complications. Time-limited hyperglycemia may occur prior to insulin injection, when the insulin pump is suspended, this relatively short period is not associated with diabetic ketoacidosis or long-term sequelae. During the trial subjects will undergo insulin-induced hypoglycemia which may be associated with temporary sweating, jitteriness, and not feeling well. The blood volume needed for each visit will be approximately 100 mL. The alternative is not to participate.

2. Adequacy of Protection against Risks

Recruitment and Informed Consent
We will contact adult with type 1 diabetes using our existing database of patients who have consented to be contacted about future diabetes research studies. A short description of the present study will be given to them via e-mail, phone or in-person. We will also recruit subjects at the time of their scheduled appointments. A one-page brochure will be prepared and sent to providers who have referred patients to us in the past. Information on the study will also be posted on our website, and the information provided on the brochure will also be made available to diabetes support groups in our area and at local events hosted by JDRF and CarbDM.

If a person expresses interest, then the study will be presented in detail. Informed consent will be obtained from adult participants by Dr. Lal or trained research staff under the supervision of Dr. Lal. Consent documents will discuss the potential risks of the study (time to complete study procedures, potential breach of confidentiality of data, intravenous catheter placement, intraperitoneal/subcutaneous injection, hyperglycemia, hypoglycemia and blood draws). Benefits of participating in the research (improved therapy for others with type 1 diabetes), and the alternative of not participating in the study will be discussed. The potential participant will be given time to review the consent and ask any questions in a private room. Dr. Lal will be available to discuss the consent process with participants.

Protections against Risk

Subjects will be instructed on the signs and symptoms of hypoglycemia and hyperglycemia, and the interventions we will take to correct these states during the study procedure. They will be wearing a continuous glucose sensor which will aid in the early detection of hypoglycemia between YSI measurements. Patients will be offered topical anesthetic prior to any of these procedures to reduce discomfort. Following injection and removal of intravenous catheter pressure will be applied to reduce and stop bleeding. All areas will be cleansed prior to invasive procedures and intraperitoneal injections will be carried out under sterile conditions to reduce risk of infection. For safety, intraperitoneal injections will be performed under continuous ultrasound guidance until the needle is removed and needles will be advanced only to the visceral peritoneal membrane to eliminate concern of bowel puncture. Where needed, intravenous catheter placement can be performed under ultrasound guidance as well. In addition, all subjects will be provided with the study doctor's contact information should they request any medical guidance following procedures.

A Data and Safety Monitoring Board (DSMB) will be formed consisting of 2 adult endocrinologists and an interventional radiologist working at institutions other than Stanford. The DSMB will review the protocol and procedures before the study is started, and will review the results after 5 patients have completed the three CTRU admissions. The PI will be responsible for reporting unexpected problematic events involving any aspect of the study to the DSMB, NIH and local IRB per institutional guidelines. Unanticipated problems to be assessed include adverse events, deviations from the study protocol, problems with informed consent, and confidentiality violations. The PI will report unanticipated problems to the DSMB, NIH and IRB within 3 business days of their occurrence. A significant study related adverse event (such as seizure, diabetic ketoacidosis, bowel puncture, severe bleeding or infection) would result in the study being put on hold until the DSMB has reviewed the event. The DSMB will review such cases and determine what actions must be taken to address or resolve the situation and if it is safe to continue the study.

3. Potential Benefits of the Proposed Research to Human Subjects and Others

Use of Continuous Glucose Monitor data will provide each subject individualized information about their glucose response to intraperitoneal and subcutaneous insulin. The data on subcutaneous insulin may allow optimization of active insulin time for pump therapy. Additionally, participants with long-standing diabetes will acquire data about his or her glucagon secretory capacity when insulin is delivered in a more physiologic manner. The precautions taken greatly reduce any risk to the subjects and optimize the benefits.

4. Importance of the Knowledge to be gained

Insulin replacement treatment is a significant burden with a narrow therapeutic margin, too much insulin results in acutely debilitating hypoglycemia and too little causes hyperglycemia that, over years, results in microvascular and coronary artery disease. Unfortunately, current subcutaneous insulin pharmacokinetics are too slow to provide automated meal coverage and the kinetics make it necessary to choose between hypoglycemia and hyperglycemia prevention, inevitably generating alarms when the user hits the opposite extreme. Additionally, there are many who stop using CGM or insulin pumps; there reasons for discontinuation include: alarms, trust in the technology and dislike of wearing devices. An implanted "invisible" pump could deliver intraperitoneal insulin with more rapid pharmacokinetics providing full closed-loop glucose control and thereby reduce the burden and morbidity of diabetes.

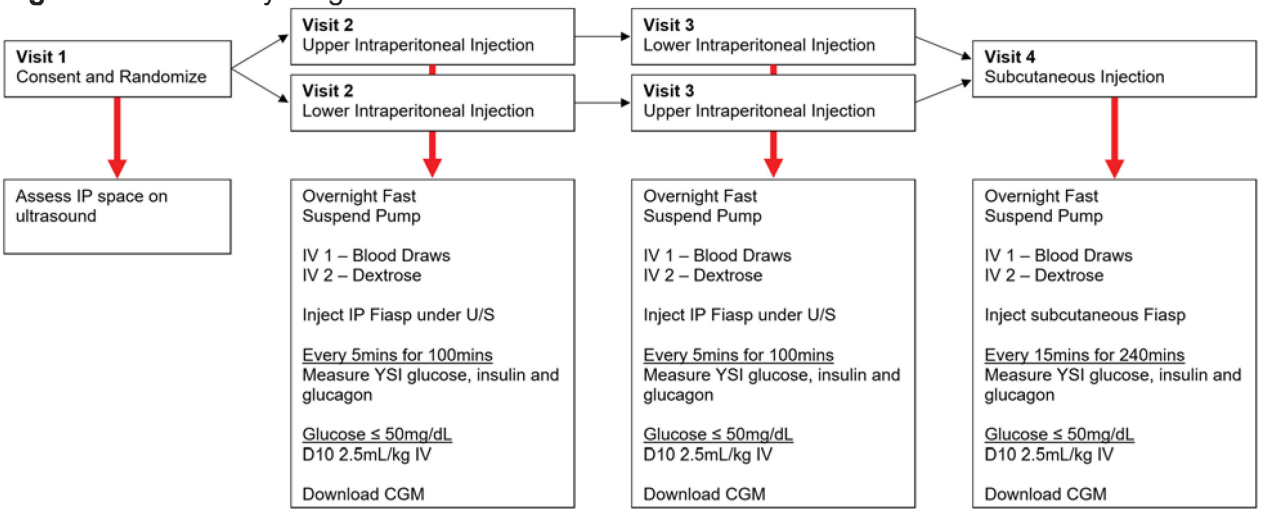
In order to test control algorithms for full closed-loop control we will need a description of insulin kinetics in the intraperitoneal space, as well as counter-regulatory hormonal factors that could counter hypoglycemia. All prior studies have used only concentrated regular insulin, which may be too slow to provide full closed-loop insulin delivery without meal announcement. Further, Dr. Eric Appel at Stanford University has recently developed excipients that stabilize newer concentrated insulin analogs. The pharmacokinetics of fast-acting insulin aspart (Fiasp) is untested in the intraperitoneal space. Another important benefit of intraperitoneal insulin is restoration of glucagon response, which has been reported in a small number of intraperitoneal insulin users.

The risks of the current study are minimal, with multiple safeguards in place. Data from the current study will directly inform a model that can be abstracted to a wide array of simulated patients. Findings from the current study will be important for the future study and development of intraperitoneal closed-loop technology. Such technology may be useful for a large number of patients utilizing insulin.

DATA AND SAFETY MONITORING PLAN

This is a single-site descriptive pharmacokinetic study comparing glucagon response to hypoglycemia for insulin Fiasp delivered via upper peritoneal, lower peritoneal and subcutaneous routes on three separate visits separated by at least one week. Demographic data and venous blood samples will be collected including glucose determined by YSI, and insulin/glucagon measured by ELISA. Following hypoglycemia :550mg/dL, patients will receive intravenous dextrose. See **Figure 1** for study diagram. The primary outcome will be glucagon response to induced hypoglycemia and a secondary outcome will be descriptive pharmacokinetics. 15 healthy adults with type 1 diabetes on insulin pump and continuous glucose monitor will be consented and patient demographic data collected. Dr. Lal or a research assistant will consent subjects in a private room with sufficient time allocated to answer any questions.

Figure 1. SA2 Study Diagram



Before any other invasive procedure the abdominal area will be assessed by an experienced interventional radiologist with gray-scale and color Doppler ultrasound to determine the safest puncture site. If the experienced interventional radiologist feels no safe area exists, the subject will be excluded prior to any invasive procedures. Subjects will perform the more difficult intraperitoneal interventions first, in random order, to avoid unnecessary procedures. If intraperitoneal access is unsuccessful, the subject will be excluded. To avoid the possibility of even minor bowel puncture, we will only take the needle tip to the edge of the visceral peritoneum, use a smaller needle and continue ultrasound recording until the needle is removed. The procedure to access the intraperitoneal space is derived from that used to place intraperitoneal catheters for peritoneal dialysis. These widespread techniques are performed safely at many health centers.

A Data and Safety Monitoring Board (DSMB) will be formed consisting of 2 adult endocrinologists and an interventional radiologist working at institutions other than Stanford. The DSMB will review the protocol and procedures before the study is started, and will review the results after 5 patients have completed the three CTRU admissions. Dr. Lal, affiliated research staff and DSMB, will periodically perform data and safety

monitoring on an ongoing basis. During the course of the trial, data will be reviewed weekly for completeness and accuracy. Frequent reviews will be performed to examine the occurrence of adverse events and whether participants are satisfied with their participation. The DSMB will be responsible for evaluating each unanticipated problem and determining whether it affects the risk/benefit ratio of the study and whether modifications to the protocol and consent forms are required. The PI will be responsible for reporting unexpected problematic events involving any aspect of the study to the NIH, DSMB and local IRB per institutional guidelines. Unanticipated problems to be assessed include adverse events, deviations from the study protocol, problems with informed consent, and confidentiality violations. Secure communication between the study team and participants will occur by telephone. All study samples will be de-identified, data will be password protected and encrypted. The PI will report unanticipated problems to the NIH, DSMB and IRB within 3 business days of their occurrence. A significant study related adverse event (such as seizure, diabetic ketoacidosis, bowel puncture, severe bleeding or infection) would result in the study being put on hold until the DSMB has reviewed the event. The DSMB will review such cases and determine what actions must be taken to address or resolve the situation and if it is safe to continue the study.

OVERALL STRUCTURE OF STUDY TEAM

The principal investigator for the study will be Dr. Rayhan Lal. Dr. Avnesh Thakor, an experienced interventional radiologist and Dr. Victoria Young (diagnostic radiologist) will be performing intraperitoneal injection under ultrasound guidance. The study will take place in Stanford's Clinical and Translational Research Unit, staffed by specialized research nurses familiar with diabetes study protocols, who will place IVs and perform blood draws. A research assistant will be in charge of enrolling, contacting and scheduling subjects. YSI glucose measures and insulin/glucagon ELISAs will be performed either by Dr. Lal or qualified study personnel.

Section 4 - Protocol Synopsis (Study 1)

4.1. Brief Summary

This will be a descriptive pharmacokinetic study of intraperitoneal versus subcutaneous Fiasp, with assessment of glucagon response to insulin-induced hypoglycemia.

4.2. Study Design

4.2.a. Narrative Study Description

15 healthy adult participants with type 1 diabetes on insulin pump and continuous glucose monitor will be consented and asked to come in for three visits separated by at least one week. Before any other invasive procedure, the abdominal area will be assessed by an experienced radiologist with gray-scale and color Doppler ultrasound to determine the safest puncture site. Gray-scale sonography will be used to find the area that provides the most separation between the anterior abdominal wall and the bowel loops. If the radiologist feels no safe area exists, the subject will be excluded.

Participants will fast overnight and suspend subcutaneous insulin delivery 3 hours prior to each procedure. For the first two visits, participants will be randomized to either upper or lower peritoneal injection. An IV will be inserted for blood draws for YSI glucose, insulin, and glucagon levels. Another IV will be placed for dextrose administration. Color Doppler sonography of the puncture site will be performed to visualize epigastric vessels and verify that they are at least 1cm away from the determined puncture site. Under sonographic guidance, a 22-gauge needle is inserted at the selected site with the needle angled 45° caudally. Real-time visualization of the needle insertion through the subcutaneous tissue, rectus abdominis muscle, and peritoneum will be performed. The peritoneum is seen as an echogenic line just deep in relation to the rectus abdominis muscle fibers. The needle will be taken to the edge of this echogenic line which may be associated with minimal momentary discomfort. Approximately 0.25u/kg Fiasp will be administered through the needle and the needle removed. Following injection, blood collection and measures will occur every 5 minutes for 150 minutes or until glucose stabilizes**.** A 10% dextrose bolus of 2.5ml/kg will be provided following a blood sugar reading ≤50mg/dl. After 150 minutes have elapsed or glucose remains stable within 10mg/dl for 2 time points, the patient will resume their home insulin regimen. Data from the patient's CGM will be downloaded.

At least one week from the last peritoneal injection a similar subcutaneous procedure will take place. An IV will be inserted for blood draws for YSI glucose, insulin, and glucagon levels. Another IV will be placed for dextrose administration. Approximately 0.25 units/kg Fiasp will be administered by subcutaneous injection. Following injection, blood collection and measures will occur every 15 minutes for 6 hours or until glucose stabalizes. A 10% dextrose bolus of 2.5ml/kg will be provided following a blood sugar reading ≤ 50mg/dl. After

4 hours have elapsed and glucose remains stable within 10mg/dl for 2 time points, the patient will resume their home insulin regiment. Data from the patient's CGM will be downloaded.

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description
Primary	Glucagon Response to Induced Hypoglycemia	Determined during upper peritoneal, lower peritoneal and subcutaneous infusion of insulin	Patient's will be given insulin via upper peritoneal, lower peritoneal and subcutaneous routes sufficient to induce hypoglycemia. We will measure change in glucagon response for venous glucose ≥50mg/dl
Secondary	Descriptive pharmacokinetics	Determined during upper peritoneal, lower peritoneal and subcutaneous infusion of insulin	Insulin levels will be measured following upper peritoneal, lower peritoneal and subcutaneous insulin administration to better characterize the kinetics of intraperitoneal insulin administration.

STATISTICAL DESIGN AND POWER

Using analyses appropriate for paired measures, we will employ paired t-tests or the analogous non-parametric test (e.g., Wilcoxon signed-rank) to assess differences in pharmacokinetic features between delivery mechanisms. In the prior 2000 study by Oskarsson and colleagues, 7 subjects with longstanding type 1 diabetes receiving subcutaneous and intraperitoneal insulin underwent induced hypoglycemia with IV insulin and had glucagon measured at baseline and during hypoglycemia. The maximal glucagon response subtracted from baseline was 7.5 ± 3.0 pg/ml on subcutaneous insulin and 17.0 ± 3.1 pg/ml on intraperitoneal insulin. Assuming a type I error rate of 0.05, 15 individuals will provide >90% power to detect an effect size of 1.0 (maximal change in glucagon of 3.0).