Equivalence of a Stable Liquid Glucagon Formulation with Freshly Reconstituted Lyophilized Glucagon

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I. Background and Significance

I. a. Background

Glucagon antagonizes the effects of insulin in the liver inhibiting glucose uptake from the blood and promoting glycogenolysis. High doses of glucagon can turn the liver into a net glucose producing organ even in the face of high serum insulin levels. Glucagon is used clinically in the treatment for life-threatening hypoglycemia when the patient cannot take oral carbohydrates. In this context it is given by subcutaneous or intramuscular injection. It is also being delivered experimentally along with insulin by a bi-hormonal bionic pancreas for the automated control of blood glucose [1-3].

Glucagon is currently available only in lyophilized form and must be reconstituted immediately before use. This process involves several steps that may be difficult for a lay person to navigate during a medical crisis, and is a barrier to the timely use of glucagon in the treatment of severe hypoglycemia.

In the context of the bi-hormonal bionic pancreas, the glucagon is delivered by subcutaneous infusion through an insulin pump. However, glucagon is chemically unstable once reconstituted, forming amyloid fibrils, becoming deamidated, and even suffering hydrolytic peptidolysis (4-6). Despite these chemical changes, the glucagon solution maintains its anti-hypoglycemic effects for at least five days (7). The U.S. Food and Drug Administration (FDA) have approved an Investigational New Drug (IND) Exemption for the use of glucagon for injection for up to 27 hours in an insulin pump in the context of bionic pancreas research.

The treatment of severe hypoglycemia in the field would be facilitated by the availability of a stable, liquid glucagon formulation that could be packaged in an automated injector, similar to an EpiPen. Likewise, commercialization of a bi-hormonal bionic pancreas will require the availability of a pump-able liquid formulation of glucagon that is stable for two to three days in an insulin pump reservoir.

Several companies have developed a stabilized liquid formulation of glucagon to meet the need. One successful approach to stabilization, taken by Xeris Pharmaceuticals (Austin, Texas), was to solubilize the glucagon in the non-aqueous solvent dimethyl sulfoxide (DMSO). In addition to providing a high degree of stability (stable at room temperature for at least nine months), glucagon is very highly soluble in DMSO so that the concentration of glucagon in this formulation can be five-fold higher than the final reconstituted concentration of currently available glucagon formulations (8-9, Appendix A). DMSO is listed by the FDA as an inert ingredient and is used as a solvent for several approved drugs, so the approval pathway for glucagon formulated in DMSO could potentially be expedited.

I. b. Pre-clinical Studies

Pre-clinical studies of Xeris glucagon have been performed in a porcine model of insulin deficient diabetes (9). Xeris glucagon was compared to freshly reconstituted lyophilized glucagon (Lilly and Co., Indianapolis, Indiana) in a bioassay of the anti-hypoglycemic effects of glucagon. Yorkshire swine were treated with streptozotocin to destroy their beta cells. The insulin deficient swine were treated with subcutaneous insulin to stabilize their blood glucose (BG) in the normal range. Lilly glucagon or Xeris glucagon (10 mcg, subcutaneous injection) was then administered and blood samples were obtained every 5 minutes for 30 minutes. After re-stabilization of the BG, the other formulation was administered. There was no difference in the rise in BG after glucagon administration (ΔBG_{max}) and no difference in the mean time to maximal blood glucagon levels (t_{max}) between Lilly and Xeris glucagon formulations (9.25 ± 4.6 vs. 10.2 ±3.6 min, p = 0.73) (Appendix B). There were no local complications associated with subcutaneous administration of either formulation.

I. c. Previous Clinical Studies

As part of a study examining the pharmacokinetics of insulin and glucagon after subcutaneous injection vs. intradermal injection, we performed subcutaneous injections of 50 mcg Lilly glucagon in 10 subjects and measured plasma glucagon levels every five minutes for 55 minutes after injection. The mean t_{max} ± SD was 16.8 ± 3.52 minutes (Appendix C). The somewhat slower absorption of glucagon in human subjects relative to swine is consistent with similar findings for insulin lispro, which is also absorbed more quickly in swine.

I. d. Rationale and Potential Benefits

Xeris Pharmaceuticals is currently conducting a study under Investigational New Drug (IND) 115091 using their non-aqueous synthetic glucagon formulation to examine safety, pharmacokinetics and efficacy as compared to Lilly glucagon in healthy volunteers. Subjects in this study received 0.5 and 1.0 mg doses of Xeris glucagon, compared to 1.0 mg of Lilly glucagon. Pharmacokinetics data is not yet available. Blinded clinical safety data has shown that all adverse events were mild to moderate in nature, and known effects of rescue doses of glucagon. In addition, Xeris recently received approval of IND 119733 to test mini-dose glucagon, with doses up to 0.3 mg. See the Investigator's Brochure in Appendix A for more information.

The proposed study is to compare the pharmacokinetics and pharmacodynamics micro-doses (0.05 and 0.03 mg) of freshly reconstituted Lilly glucagon with Xeris glucagon in healthy volunteers with type 1 diabetes. The hyperinsulinemic-normoglycemic clamp protocol is the standard method to perform pharmacokinetic and pharmacodynamics studies on drugs that effect BG. The doses of glucagon chosen for this study are in the range of doses that are routinely given by the bi-hormonal bionic pancreas. They are expected to result in a modest but easily measureable decrease in the glucose infusion rate (GIR), and will be directly relevant to infusion in the context of a bi-hormonal bionic pancreas. The direct comparison of Lilly glucagon (which has been used in all of the bi-hormonal bionic pancreas studies to date) and Xeris glucagon will provide the

preliminary data required to obtain FDA evaluation of Xeris glucagon in future bionic pancreas studies.

II. Hypothesis and Specific Aims

We hypothesize that Xeris glucagon will be non-inferior to freshly reconstituted Lilly glucagon both in terms of pharmacokinetics (t_{max}, $t_{½max}$) and pharmacodynamics (AUC_{GIR}), and in terms of safety. The specific aims of this study are:

Aim 1. To compare the pharmacokinetics and pharmacodynamics of Lilly and Xeris glucagon after low-dose subcutaneous injection using the hyperinsulinemic-normoglycemic clamp technique in volunteers with type 1 diabetes.

Aim 2. To document any adverse events of the Xeris glucagon formulations after subcutaneous injection in volunteers with type 1 diabetes.

III. Subject Selection

III. a. Inclusion Criteria

- Age 21 to 80 years old with type 1 diabetes for at least one year.
- Diabetes managed using an insulin infusion pump using rapid-acting insulin such as insulin aspart (NovoLog), insulin lispro (Humalog), or insulin glulisine (Apidra) for at least one week prior to enrollment.

III. b. Exclusion Criteria

- Unable to provide informed consent.
- Unable to comply with study procedures.
- Current participation in another diabetes-related clinical trial that, in the judgment of the principle investigator, will compromise the results of the clamp study or the safety of the subject.
- Pregnancy (positive urine HCG), breast feeding, plan to become pregnant in the immediate future, or sexually active without use of contraception.
- End stage renal disease on dialysis (hemodialysis or peritoneal dialysis).
- History of pheochromocytoma. Fractionated metanephrines will be tested in patients with a history increasing the risk for a catecholamine secreting tumor:
    - Paroxysms of tachycardia, pallor, or headache. Personal or family history of MEN 2A, MEN 2B, neurofibromatosis, or von Hippel-Lindau disease
    - Episodic or treatment of refractory (requiring 4 or more medications to achieve normotension) hypertension.
- History of adverse reaction to glucagon (including allergy) besides nausea, vomiting, or headache.
- Inadequate venous access as determined by study nurse or physician at time of screening.

- Liver failure or cirrhosis.
- Hemoglobin < 12 gm/dl.
- Any other factors that, in the judgment of the principal investigator, would interfere with the safe completion of the study procedures.

No volunteers will be excluded on the basis of gender or race. The requirement that volunteers manage their diabetes using subcutaneous insulin infusion pump therapy is imposed because multiple daily injection therapy involves the use of medium-acting basal insulin (such as NPH insulin) or long-acting insulin (such as glargine) that would either require an extended washout period or would result in unwanted variation in the GIR due to the pharmacokinetics of longer acting insulins.

III. c. Source of Subjects

Volunteers who fit the selection criteria will be considered as candidates for this study. Advertisements for the study will be posted at the MGH Diabetes Center and will be distributed in the weekly broadcast email of research studies seeking volunteers. We will post basic information about the trial along with contact information on our website www.artificialpancreas.org and the study will be posted on www.clinicaltrials.gov. We will also post information regarding the trial at online venues for people with type 1 diabetes, such as Glu (online community of the Type 1 Diabetes Exchange).We will also contact individuals who have previously inquired about participation in our studies and have asked us to have their contact information kept on file.

IV. Subject Enrollment

IV. a. Number of Subjects

It is expected that we will have 12 volunteers complete full-length clamp experiments using a consistent protocol and that the experiments can be accomplished over a period of 4-6 months.

Up to 30 volunteers with type 1 diabetes will be enrolled. The upper bound is based on the expectation that some volunteers will be excluded after the screening visit and blood tests, the possibility that some qualified volunteers will not be able to complete an experiment for scheduling reasons, the possibility that some experiments may have to be discontinued before completion (e.g. due to inability to maintain IV access or subject withdrawal), and the likelihood that minor modifications to the protocol for adjustment of the GIR or the insulin infusion rate may be required during the first 2-3 experiments. Thus, it is likely that 15 clamp experiments will need to be performed to complete 12 experiments with a consistent protocol.

IV. b. Enrollment Procedures

Prospective participants may be briefed by a study staff member by phone regarding the study procedure and the inclusion and exclusion criteria. Potential volunteers may be sent an informed consent document to review by email or post.

IV. c. Consent Procedures

Potential volunteers will meet with a study physician or nurse practitioner who will explain the study and answer questions. Informed consent will be administered by an MD or NP. In the event that a volunteer is a patient of one of the study MDs or NPs, another staff MD or NP will answer questions and administer consent. If a nurse practitioner is administering the consent, a physician will be available as back-up for additional support if needed and subjects will be offered the chance to speak with a study physician if they wish.

The study physician or nurse will also answer any questions that the volunteer may have during their participation. They will share any new information in a timely manner that may be relevant to the volunteer's willingness to continue participating in the trial. The volunteers may choose to discontinue their participation at any time.

V. Study Procedures

V. a. Screening data

- Age
- Sex
- Race and ethnicity
- Urine HCG for female volunteers
- Date of diabetes diagnosis
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Date of last menstrual period in female volunteers
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Duration of insulin pump use
- Insulin regimen (basal rate, sensitivity factor, and carbohydrate ratio)
- Average total daily dose of insulin in the last 30 days (from pump history)
- Height and weight
- Blood pressure
- Hemoglobin A1c
- Hemoglobin
- Creatinine and estimated GFR
- Alanine aminotransferase (ALT)
- Serum albumin
- Fractionated metanephrines (in patients with history increasing the risk for a catecholamine secreting tumor)

V. b. Drugs

The study involves subcutaneous administration of *Lilly glucagon* and *Xeris glucagon.* Lilly glucagon is commercially available by prescription and is indicated for patients with type 1 diabetes. Xeris glucagon has the same active pharmaceutical ingredient, but is dissolved in a different vehicle at a higher concentration. Xeris glucagon in dissolved in a non-aqueous solvent that is composed primarily of dimethyl sulfoxide (DMSO) at a concentration of 5 mg/ml, while Lilly glucagon is dissolved in an aqueous vehicle at a concentration of 1 mg/ml. DMSO is listed by the FDA as an inert ingredient and is a component of several approved drug formulations. Xeris glucagon is stable at room temperature for greater than one year (see the Investigator Brochure in Appendix A). The age of the Xeris glucagon at the time of administration will depend on when the experiments are done, but we will complete all of them within the already determined window of stability. Based on the date of manufacture of the current lot, we expect the studies to be done with material that is approximately 6 months old (between 5 and 7 months old).

The clamp procedure involves intravenous administration of *regular human insulin*, which is commercially available without a prescription and is indicated for patients with type 1 diabetes. The insulin infusion includes 2% *human serum albumin*, which is added to the solution to prevent inactivation of insulin or loss of insulin on the walls of the administering syringe and IV tubing (10-11). The clamp procedure also involves intravenous administration of *dextrose 20% solution* for infusion

V. c. Devices

Intravenous catheters: Each volunteer will have two intravenous catheters introduced. One will be dedicated to withdrawal of samples and the other for infusion of insulin and dextrose.

HemoCue Blood Glucose Meter: The HemoCue is an FDA approved blood glucose meter with lab equivalent accuracy. Blood glucose measurements will be obtained with the HemoCue meter using samples of venous blood.

Hot box: A hot box will be used to warm up the forearm and hand of the subject in which the sampling IV catheter is placed for the purpose of arterializing venous blood.

V. d. Experimental Procedures and Data Collection

Screening Visit

- All volunteers will have a screening visit to confirm eligibility
- Female volunteers will have urine tested for HCG. If the test is positive, the volunteer will be informed of the result and the visit will be ended.

- The volunteer will be interviewed and the case report form will be completed by a study nurse or study physician to establish whether the volunteer is eligible to continue with the screening.
- A blood draw will be performed to test hemoglobin, and for metanephrine screening, if indicated. A study physician or nurse practitioner will review the case report form and laboratory results to determine volunteer eligibility. If volunteers are not eligible to continue in the study, the results of abnormal tests will be reported to the volunteers and to a health care provider of their choosing.

Hyperinsulinemic-normoglycemic clamp

- Subjects will report to the Clinical Research Center (CRC) by 7:00 AM in the fasted state.
- Two intravenous (IV) catheter lines will be placed, one in each arm. The sampling IV will be placed on the dorsal surface of the hand or the forearm. The catheters will be 20 gauge or smaller in diameter.
- Blood will be drawn for creatinine, ALT, albumin, and hemoglobin A1c.
- Regular human insulin will be added to this solution of 2% human serum albumin in normal saline to allow an infusion rate of 5-20 ml/hr based on the desired hourly insulin dose. Mixing of the insulin infusate will be performed by the research pharmacy and the final desired insulin concentration will be communicated to the research pharmacy in advance of the visit. The range of insulin concentrations in the infusate are expected be from 0.2-1.2 u/ml, depending on the mean basal insulin rate of the subject.
- The subject will remove their own insulin pump and a primed continuous insulin infusion will be started using the insulin/saline/albumin mixture. The continuous rate will be between two-fold and four-fold higher than the mean basal insulin rate of the subject. The insulin infusion may be used to give insulin boluses if the subject is hyperglycemic at arrival to reduce the time necessary to reach steady state. Once the appropriate rate is established (with a dextrose infusion rate that is neither too low nor too high), it will remain stable throughout the experiment. For a 70 kg, 5'10" tall man with a usual basal rate of 1 u/hr, an infusion at two-fold the usual basal rate would be 17 mU/m2/min. Therefore, for a 5 ml/hr infusion 19.88 units of insulin would be added to 50 ml of saline/albumin solution (~0.4 u/ml).
- An infusion of 20% dextrose in water will be started. BG values will be measured every five minutes using arterialized blood measured with the HemoCue. The dextrose infusion will be adjusted so that the blood glucose is stabilized at 90 ± 5 mg/dl. This is expected to take approximately two hours. If the dextrose infusion rate is less than 2 mg/kg/min or greater than 8 mg/kg/min then the insulin infusion rate may be adjusted. For the 70 kg, 5'10" tall man this would correspond to 42-168 ml/hr. If this process takes longer than 3 hours, the study will be stopped.
- Blood samples (2 ml) will be processed for plasma for later insulin and glucagon measurement every 20 minutes throughout the experiment.

- Once the insulin infusion rate is established and the dextrose infusion rate has been stable for 20-30 minutes with the BG in the target range, two baseline plasma samples will be obtained and the first glucagon injection will be given.
- The order of glucagon injections will be randomized and double blinded. Neither the subject nor the provider running the experiment will know what each injection is. The study nurse will prepare the medications and label the syringes "1" and "2", for the order of injection.
- An injection of 30 mcg of Lilly glucagon or 50 mcg of Xeris glucagon (the order will be determined by block randomization) will be administered by subcutaneous injection using a syringe and a small gauge needle at a depth of less than 1 cm.
- In the 60 minute period after the glucagon injection, the frequency of BG checks will be increased to every two minutes and blood samples will be processed for plasma at each check. After this 60 minute period, the sampling interval will return to 5 minutes and blood samples will be processed for plasma every five minutes until the GIR has stabilized for three samples, when the sampling interval will be reduced to every 20 minutes.
- The dextrose infusion rate will be adjusted to maintain the BG in the target range. Since glucagon antagonizes the effects of insulin in the liver, the dextrose infusion rate will be reduced and then increased again to baseline as the glucagon effect waxes and wanes.
- The injection site will be examined every five minutes for the first 15 minutes after injection and then every 15 minutes until 1 hour after the injection. Any local reactions will be documented, including a qualitative description and measurement of any skin reaction.
    - Dermal responses will be measured after each injection using the Draize scale, which quantifies erythema and eschar, and edema on a scale of 0-4, with 0 representing none and 4 representing severe.
- Subjects will be asked to rate the pain associated with injection on a 10 cm standard visual analog scale (VAS): 0 = no pain, 10 = worse imaginable pain
- Subjects will be asked at one hour after the injection to rate their maximal nausea during the preceding hour on a VAS: 0 = no nausea, 10 = vomiting
- Given what is known about the pharmacokinetics of glucagon given by the subcutaneous route, it is expected that it will take 60-90 minutes for the dextrose infusion rate to stabilize again.
- Once the dextrose infusion rate has remained stable for 20-30 minutes with the BG in the target range, the second glucagon injection will be given.
- A 30 mcg injection of Lilly glucagon or 50 mcg injection of Xeris glucagon (whichever formulation was not given in the first injection) will be administered by the subcutaneous route using a syringe and a small gauge needle at a depth of less than 1 cm.
- In the 60 minute period after the glucagon injection, the frequency of BG checks will be increased to every two minutes and blood samples will be processed for plasma at each check. After this 60 minute period, the sampling interval will return to 5 minutes and blood samples will be processed for plasma every five minutes until the GIR has stabilized for three samples, when the sampling interval will be reduced to every 20 minutes.

- The dextrose infusion rate will again be adjusted to maintain the BG in the target range. The dextrose infusion rate will be reduced and then increased again to baseline as the glucagon effect waxes and wanes.
- It is expected that it will take 60-90 minutes for the dextrose infusion rate to stabilize again.
- Once the dextrose infusion rate has remained stable for 20-30 minutes with the BG in the target range, the experiment will be ended and the insulin infusion will be stopped.
- The subject's insulin pump will be replaced and restarted at the end of the experiment.
- The subject will be given a small meal. The provider will help the patient determine how much of their own insulin (from their insulin pump) they should bolus, if any.
- The dextrose infusion will either be stopped or titrated down by the provider.
- Samples will be drawn PRN for a HemoCue blood sugar until the subject's BG has remained stable for 30 minutes or as determined stable by the provider.
- Both of the IV catheters will be removed.
- The total clamp time is expected to be five to six hours, but could potentially run longer if it takes longer to reach equilibrium initially or after glucagon injections.
- The total blood obtained is expected to vary from 200-300 ml. In no case will more than 400 ml of blood be taken.
- Subjects will not be allowed to sleep during the clamp visit, as sleep can affect glucose metabolism. Ambulating to the bathroom will be permitted if it will not interfere with study procedures and the subject's blood glucose is stable. A urinal and a bedside commode will also be made available to subjects should they need it.
- Analysis of the initial experiments may lead to optimization of the protocol. After the glucagon injections, the GIR should decrease by at least 20% but should not be reduced to zero at any time. If the glucagon dose is not sufficient to decrease the GIR at least 20%, then lower insulin infusion rates will be used. If the glucagon dose results in the GIR being reduced close to zero, then higher insulin infusion rates will be used.

V. e. Response to adverse events

Minimal nausea was noted in clinical trials of the bi-hormonal closed-loop system in which up to 80 mcg of glucagon was given at intervals of as little as 5 minutes. The mean glucagon dose in these trials was ~1 mg over 24 hours. Therefore, we do not anticipate significant nausea associated with two 30-50 mcg doses of glucagon separated by at least one hour (total dose of 80 mcg). However, nausea is a potential side effect of glucagon.

If vomiting occurs, a study physician or nurse practitioner will be notified. If in the judgment of the provider the vomiting was likely to be due to glucagon dosing, no further glucagon doses will be given. If both glucagon doses have already been given at the time of vomiting, then the experiment will be completed if the subject is willing since completing the experiment will not involve administration of more glucagon.

V. f. Supervision by Study Staff

A study physician or nurse practitioner will order all changes to the insulin infusion and glucose infusion rates and will be on site at all times once the clamp has begun and until it is ended.

VI. Biostatistical Analysis

VI. a. Data Collected

At the time of enrollment:

- Age
- Sex
- Race and ethnicity
- Urine HCG for female volunteers
- Date of diabetes diagnosis
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Date of last menstrual period in female volunteers
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Duration of insulin pump use
- Insulin regimen (basal rate, sensitivity factor, and carbohydrate ratio)
- Average total daily dose of insulin in the last 30 days (from pump history)
- Height and weight
- Blood pressure
- Hemoglobin A1c
- Hemoglobin
- Creatinine and estimated GFR
- Serum Albumin
- Alanine aminotransferase (ALT)
- Fractionated metanephrines (in patients with history increasing the risk for a catecholamine secreting tumor)

During the hyperinsulinemic-normoglycemic clamp:

- Venous BG measurements
- Timing and size of glucagon doses
- Insulin infusion rate
- Glucose infusion rates
- Plasma insulin levels every 15-30 minutes during the hyperinsulinemic-normoglycemic clamp

- Plasma glucagon levels every 2-15 minutes during the hyperinsulinemic-normoglycemic clamp
- Any adverse events, including nausea, vomiting, pain, and any local reactions to glucagon injection

VI. b. Study Endpoints

Primary endpoint analysis:

- Glucagon t_{max} for Xeris vs. Lilly (non-inferiority)

Secondary endpoint analyses:

- Area over the curve for glucose infusion rate in the hour following a subcutaneous glucagon dose (AOC_{GIR}) for Xeris vs. Lilly
- Maximal glucose infusion rate (GIR_{max}) for Xeris vs. Lilly
- Glucagon $t_{½max}$ for Xeris vs. Lilly
- Quantitation of adverse events related to glucagon injection for Xeris vs. Lilly:
    - Injection pain on a 10 cm standard VAS: 0 = no pain, 10 = worst imaginable pain
    - Injection site erythema or other local reaction, maximum diameter within 1 hour of injection
    - Maximal nausea within 1 hour of injection on a 10 cm VAS: no nausea = 0, vomiting = 10

We will calculate means, median, percentages, standard deviations, standard errors, inter-quartile ranges, and 95% confidence intervals in descriptive analyses. We will use paired t-test for comparison of means. We will use multivariate regression models with repeated measurements to compare means and percentages while adjusting for patient demographics characteristics such as age, gender, body mass index, and body surface area.

VI. c. Power Analysis

Our previous clinical study found a mean $t_{max} \pm$ SD for Lilly glucagon after subcutaneous injection of 50 mcg of 15.8 ± 3.52 min (Appendix C). A previous pre-clinical PK/PD study in diabetic swine found no significant difference in t_{max} between Lilly and Xeris glucagon (Appendix B). This study is powered to achieve 90% power to determine non-inferiority of Xeris vs. Lilly glucagon t_{max} using a one-sided t-test when the margin of equivalence is 2.52 minutes (15% of the t_{max} for Lilly glucagon in humans) and the true difference between the means of Lilly and Xeris glucagon is assumed to be 0. Therefore, the null hypothesis is that the tmax of Xeris glucagon is not worse than the tmax of Lilly glucagon by more than 2.52 minutes. The assumed standard deviation for each glucagon is 3.52 based on the clinical data. We assume a correlation coefficient between Lilly and Xeris glucagon within the same subject to be 0.7 in this crossover design, resulting in an SD of 2.73 in the difference between the two. The significance level (alpha) of the test is

0.05. The sample size required to achieve the designated power is 12 clamp experiments, each including one injection of Lilly glucagon and one injection of Xeris glucagon.

VII. Risks and Discomforts

There is a potential risk of nausea or vomiting in volunteers due to the administration of exogenous glucagon. The experiments, however, involve small subcutaneous glucagon doses. The recommended dose of glucagon for treatment of severe hypoglycemia in an adult with diabetes is 1000 mcg, given as a single subcutaneous or intramuscular injection. In practice, a smaller dose of 500 mcg is sometimes used initially to reduce the risk of nausea and vomiting. The total dose to be administered in our study is 100 mcg.

There is a potential risk of hypoglycemia, since exogenous insulin will be administered. Given frequent BG monitoring (every two to five minutes), corresponding adjustment of a continuous dextrose infusion, and direct supervision by an NP or MD at all times, the risk of a hypoglycemic episode leading to significant harm to volunteers is expected to be very low. A small meal will be provided to subjects after completion of the study.

There is a theoretical risk of infection associated with the use of albumin, a protein purified from donated blood, The risk of transmitting disease is reduced by testing blood donors for infections, and by heat treating and purifying the albumin. Because of these measures, the risk is considered to be very small; no cases of disease transmission have every been identified for albumin.

The risks of intravenous lines remaining in place for about10 hours include thrombosis and phlebitis of the peripheral vein.

Subjects may experience discomfort with insertion of the peripheral intravenous line. Subjects may also experience mild discomfort associated with the subcutaneous injections of glucagon.

There is a risk of risk of dizziness or lightheadedness form blood loss. However, typical blood loss will be ~200 ml and loss of blood will be limited to no more than 400 ml.

VIII. Potential Benefits

The data derived from this study will allow us to validate the use of a stabilized glucagon formulation. Eventual commercial availability of this formulation will expand treatment options and reduce the risk of complications from hypoglycemia for all patients with diabetes. There is no direct benefit for the subject participating in this study.

IX. Data and Safety Monitoring

IX. a. Monitoring of Source Data

The principal investigator (PI), a study clinical research fellow (physician), or a study nurse practitioner will review the eligibility of each volunteer based on the case report from the screening visit.

All data from study visits will be combined in a single database that will be compared against the primary data files for integrity. The computer database will be backed up at least monthly and the backup media stored in a secure location.

The study will be conducted by the staff of the MGH Diabetes Research Center (DRC) in cooperation with the staff of the CRC. The PI will conduct meetings with study staff at least twice a month to review study progress, discuss any issues in study conduct, and review procedures. Study staff will be encouraged to raise any concerns they may have or problems they have identified at these meetings. The PI will decide a course of corrective action, and resolution or progress will be assessed no later than the next bimonthly meeting. An audit of procedures, regulatory documentation, and a sample of volunteer files will be performed by a member of the DRC at least biannually. The audit will be conducted by a DRC staff member not directly involved in the conduct of the study. This audit will include a review of regulatory documentation, such as IRB correspondence, and a review of files, including a review of consents, case report forms, and other data from study visits.

A numeric code will be substituted for the volunteers personal identifying information in the study database, which will be password protected. The key linking the medical record number of the volunteer with the numeric code, along with case report forms, and all information that is personally identifiable, will be kept in a locked filing cabinet in an investigator's locked office. All electronic records will be kept in a password protected computer database. All printed computer data will be disposed of confidentially when no longer needed. Only the study staff will have access to the study database. Subjects may not withdraw from the de-identified database, but they may elect to have the key linking their medical record to the de-identified database destroyed.

The study data may be shared with collaborators outside of Partners, but only in a form in which all personally identifiable information has been removed. Shared data will be in the form of a database in which only a number identifies volunteers.

IX. b. Safety Monitoring

A study physician or nurse practitioner will directly supervise each experiment. The PI will be informed of any adverse events immediately and will make any adjustments to the study protocol as needed to maintain subject safety.

The Research Pharmacy will mix the insulin solution. The calculations for the insulin infusion rate and the mixing of the insulin solution will be done in advance and checked by another physician or nurse practitioner.

This study will be conducted under an Investigational New Drug application sponsored by the PI.

This study is considered moderate risk. The Principal Investigator and co-investigators will evaluate each experiment. Unanticipated problems, including adverse events, will be reported to the Partner's IRB in accordance with the PHRC policy on Unanticipated Problems Involving Risks to Subjects or Others including adverse events. An external Data and Safety Monitoring Board will oversee the conduct of the study and review its results on a regular basis. Additionally, the DSMB will be informed after any experiment that has to be discontinued due to hypoglycemia, hyperglycemia, or any unexpected adverse event. DSMB review will occur before any further experiments are performed. The DSMB will be informed if there are any changes to the study protocol that could significantly impact the safety or scientific validity of the study. A final DSMB meeting will convene after the completion of the study. After the first DSMB meeting, subsequent meetings may be convened via e-mail or conference call. Safety and efficacy data will also be reported to the FDA in compliance with applicable regulations.

IX. c. Adverse Event Reporting Guidelines

The PI will review any adverse events after each experiment. Adverse events will be reported to the Partner's IRB, the DSMB, and to the FDA.

X. Subject Compensation

Financial compensation of $50 will be provided to all subjects who complete a screening visit. Subjects will be compensated $250 for participation in the hyperinsulinemic-normoglycemic clamp study visit.

XI. References

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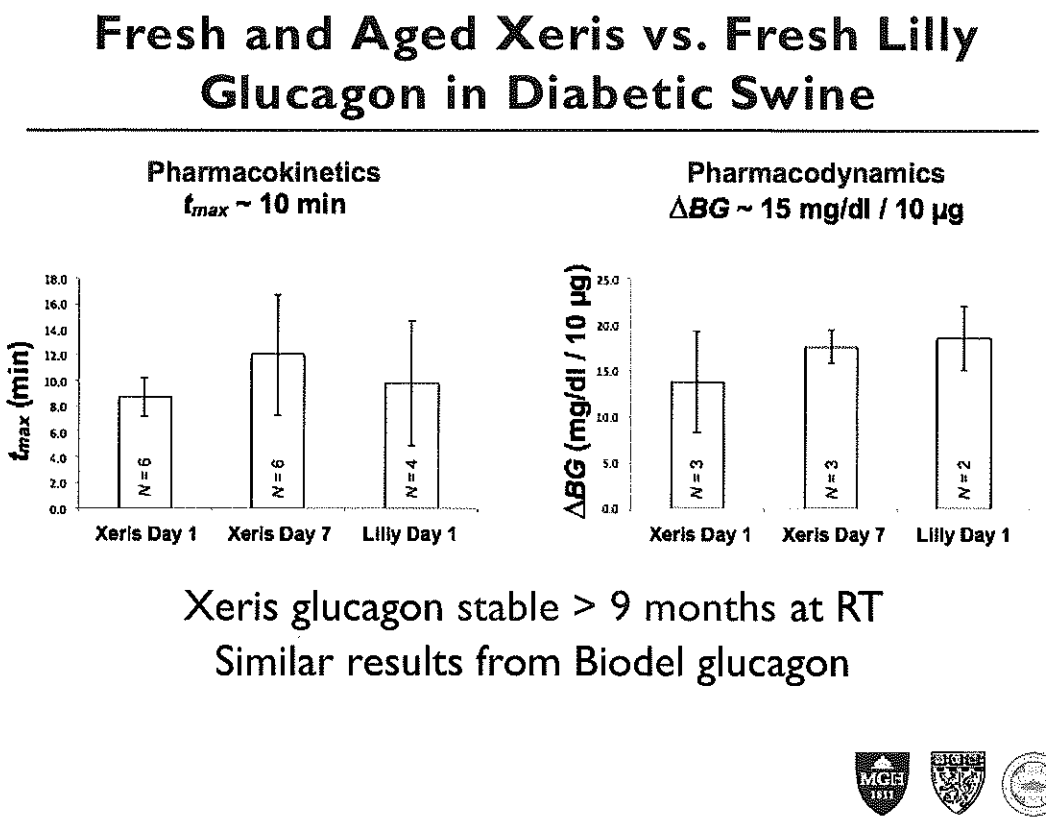
Appendix A. Investigators brochure for G-pen glucagon.

Appendix B. Pharmacokinetics and pharmacodynamics of Xeris vs. Lilly glucagon after subcutaneous administration in diabetic swine.



Appendix C. Pharmacokinetics of subcutaneous microdose Lilly glucagon in human subjects with type 1 diabetes.

Subject ID	**Experiment**	**Glucagon tmax (min)**
102	1st	12
102	2nd	15
103	1st	11
103	2nd	20
105	1st	15
105	2nd	19
106	1st	21
106	2nd	16
108	1st	19
108	2nd	20
Mean		16.8
SD		3.521363372