Role of alpha-cell GLP-1 in the beta-cell response to DPP-4 inhibition

BACKGROUND AND SIGNIFICANCE: GLP-1 is a potent insulin secretagogue that has a critical role in the incretin effect. The conventional model of GLP-1 action is that it acts as a hormone, released from enteroendocrine cells into the circulation during nutrient absorption, mediating its effects directly on target tissues like the β-cell. However, this model has been questioned because of the relatively small dynamic range of postprandial GLP-1 secretion, and its rapid inactivation by DPP-4. We have recently reported that in mice GLP-1 signaling is necessary for normal glucose tolerance, but this effect is independent of circulating GLP-1. Moreover, in studies of healthy and diabetic humans we have observed that blockade of the GLP-1 receptor (GLP-1r) reduces glucose-stimulated insulin secretion in the fasting state when plasma active GLP-1 levels are low and unchanging. These findings are consistent with an emerging body of work questioning an endocrine model of GLP-1 action. One hypothesis advanced as an alternative is that GLP-1 produced in islet α-cells acts in a paracrine manner to regulate neighboring β-cells. This model is supported by several recent reports demonstrating islet production and action of GLP-1. In fact, evidence from rodent studies, and from our work in humans, indicates that there is a greater effect of islet GLP-1 signaling during periods of increased β-cell demand. Taken together, the published literature raises the possibility that intra-islet GLP-1 action is a mechanism of β-cell compensation. If true, this model would dramatically shift understanding of the incretin system and present a range of new opportunities for diabetes treatment.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are an FDA-approved class of oral antihyperglycemic agents for the treatment of diabetes mellitus type 2. DPP-4 inhibitors have become a widely used drug for the treatment of diabetes, a practice driven by their ease of use, tolerability, and paucity of side effects, and they are often used in combination with other antihyperglycemic medications. It is believed that inhibition of DPP-4 prevents inactivation of GLP-1, allowing it to function more efficiently as an insulin secretagogue. However, in the absence of a clear understanding of how these drugs work (i.e. effects on plasma GLP-1 an endocrine hormone vs. effects on α-cell secretion of GLP-1 an a paracrine factor), their use is essentially empiric with no clear rationale for their use in combination with other glucose lowering agents, and no obvious direction for development of methods to augment their activity. This proposal describes experiments in humans to test the effect of DPP-4 inhibition on insulin secretion in fasted humans with low and unchanging plasma GLP-1. These studies are directed at establishing the paracrine model of islet GLP-1 action.

PURPOSE OF STUDY: This study is funded by Merck and is being led by Dr. David D'Alessio in the Division of Endocrinology and the Duke Molecular Physiology Institute. The study will address the following two specific aims:

Aim 1- To determine the roles of fasting GLP-1 and alpha-cell secretion on insulin release. In this aim we will test the effects of DPP-4 inhibition and GLP-1 blockade on glucose-stimulated insulin secretion in fasting humans with low and unchanging plasma GLP-1. These effects will be tested further under conditions of α-cell stimulation with arginine.

Aim 2- To determine the relative effect of fasting GLP-1 action in diabetic and nondiabetic subjects. We hypothesize that α-cells release greater relative amounts of GLP-1 during times of increased β-cell demand. We will compare the results from the experiment described below in groups of age- and BMI-matched diabetic and nondiabetic subjects.

DESIGN AND PROCEDURES: The study will enroll up to 70 participants to be divided evenly between two subject populations: a diabetic cohort and an age/BMI matched nondiabetic cohort. We plan to match ±2 years in age and ± 1.5 k/m2 in BMI. While we anticipate that the screening blood draw will yield a number of screen failures, we expect that approximately 40 subjects will complete the entire study protocol. All study visits and procedures

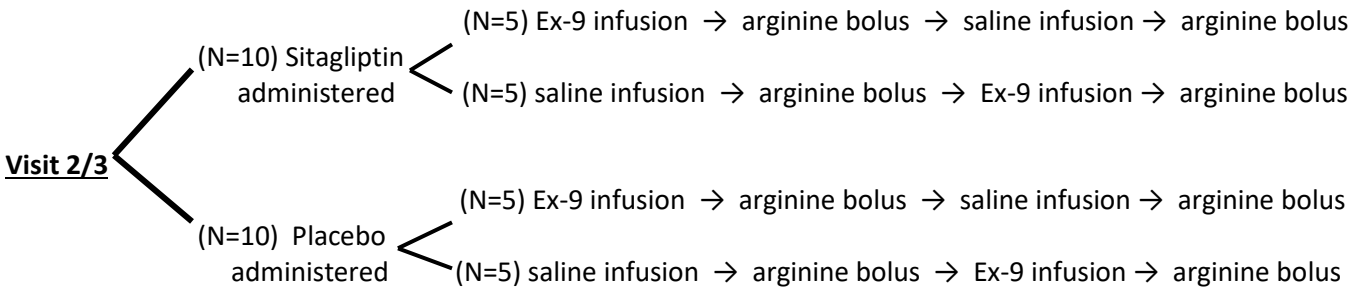
will take place in the Human Physiology Testing Core shared resource facilities located at the Duke Center for Living campus.

Visit 1: The consent visit will take place at the Duke Center for Living and will last approximately two hours. We will explain the study and the consent form in detail. Upon signing the consent form, a sample of blood (one teaspoon) will be drawn to measure the hemoglobin A1c (HbA1c), hematocrit and eGFR to ensure that the participant meets inclusion criteria. If these values are within the range listed below for the appropriate cohort, the participant will be scheduled for Visit 2.

- Diabetic Cohort – HbA1c ≤ 8.0% plus a diagnosis of type II diabetes
- Non-Diabetic Cohort – HbA1c ≤ 6.2%

Visits 2 and 3: Each participant will have two 4.5-hour infusion procedures. During one visit, the participant will receive 100 mg of oral Sitagliptin (an FDA-approved DPP-4 inhibitor) at the beginning of the procedure and at the other visit, they will receive a placebo. The order of these treatments will be counterbalanced across the cohorts such that half of each group takes Sitagliptin on the first study day. Participants in the diabetic cohort will be asked to stop their usual medications for 3 days prior to each infusion study visit. These participants will be provided guidance on how best to manage their glucose control via dietary intake over these 3 days.

Subjects will report to the Duke Center for Living after a 12-hour fast. Participants will be given either 100 mg of Sitagliptin or a placebo tablet at the beginning of the infusion procedure. Subjects will have two intravenous catheters placed, one in each forearm: 1) for blood drawing, and 2) for the infusion of test materials. The arm for blood draws will be heated to maintain blood flow and arterialize venous blood. Three fasting blood samples will be taken at -10, -5 and 0 minutes and an infusion of either Exendin-9-39 (abbreviated as Exendin-9 or Ex-9), which functions as a competitive antagonist of the GLP-1 receptor) (750 pmol/kg/min) or saline will be continuously infused for 60 minutes; blood samples will be taken at 5, 10, 15, 20, and 25 minutes. At time 30, subjects will receive a 50ml infusion of 5g arginine (a simple amino acid) over 1 minute; blood samples will be taken at 32, 34, 36, 38, 40, 42, 45, 48, 51, 55 and 60 minutes and the Ex-9/saline infusion stopped for 60 minutes. Blood samples will be taken at 110, 115 and 120 minutes and an infusion of Ex-9/saline started, subjects receiving the alternative substance to their first infusion. Blood will be sampled at 125, 130, 135, 140 and 145 minutes and the arginine bolus repeated at 150 minutes. Blood will be sampled at 152, 154, 156, 158, 160, 162, 165, 168, 171, 175, and 180 minutes and the infusion stopped, completing the experiment. At each time-point listed above, approximately 4.5ml of blood will be drawn for a total of 159ml (~11 Tbsp) of blood taken during the entire 4.5-hour infusion procedure. . If a participant becomes symptomatic, glucose will be measured using a handheld glucometer. **Visit 3** will occur at least a week later but no later than three months (i.e. approximately 90 days) following Visit 2. Visit 3 will be a repeat infusion procedure identical to **Visit 2**, but with the alternative to the Sitagliptin/placebo treatment that the participant received in the first experiment.



Using this design we will be able to assess the effect of GLP-1 and DPP-4i on:

- Fasting insulin secretion.
- Arginine stimulated insulin secretion.

Note that a skin test will be conducted to evaluate any potential sensitivity to Ex-9 prior to the infusion of the test substance on the day of their first study visit; such that the first time a subject is to receive this substance they must first have a negative skin test. There will not be a need to repeat the skin test at the second infusion procedure visit (Visit 3). The skin test is specified by the FDA as a precaution for immediate hypersensitivity; 5 ng of Ex-9 is injected intradermally and subjects are monitored for 30 minutes for a wheal/flare reaction.

SELECTION OF SUBJECTS: Study participants will be recruited using newspaper advertisements and flyers posted across Duke hospital/clinic facilities, inviting interested individuals to contact the study recruitment coordinator for additional information. Additionally, we will use the DEDUCE software to identify potential participants for the diabetic cohort.

- Diabetic Cohort – HbA1c ≤ 8.0% plus a diagnosis of type II diabetes managed by either Metformin, Sulfonylurea or diet and exercise
- Non-Diabetic Cohort – HbA1c ≤ 6.2%

- Inclusion criteria:
  Healthy adults age 35-70 years
  Male or female
  Ability to speak and understand English

- Exclusion criteria:
  Rheumatoid arthritis
  Inflammatory bowel disease
  Unstable angina or uncompensated heart failure
  Pulmonary disorders, including COPD and asthma
  Malabsorptive GI disease, such as celiac disease, or gastric bypass
  Significant hepatic disease
  Renal insufficiency (eGFR < 60 mL/kg/min)
  Anemia (hematocrit < 34%) as measured at screening visit
  Uncontrolled hypertension
  Pregnant females
  Consumption of daily medications that alter glucose metabolism of GI function (glucocorticoids, psychotropics, narcotics, metoclopramide)
  Consumption or injection of insulin
  Apparent sensitivity to any of the study peptides as determined by the skin test

Exclusion criteria will primarily be identified by way of a phone screen process conducted by the recruitment coordinator, however medical record review may be used to confirm the absence of a diabetes mellitus diagnosis or other medical conditions listed as exclusion criteria.

SUBJECT IDENTIFICATION, RECRUITMENT AND COMPENSATION: Potential subjects for this study will be directed to contact the recruitment coordinator to obtain more information about the study, if interested. When contacted, the recruitment coordinator will confirm the participant meets most of the inclusion/exclusion criteria before scheduling him/her for a consent meeting. Subjects will be included in a group informational session to provide a formal overview of study activities, conducted by the study coordinator or another designated member

of the key personnel. Following this, potential subject will be taken to a private, secure location, where the study coordinator or other designated member of the study team will answer any questions that he/she may have and will obtained informed consent. We will allow at least 30 minutes at the end of the consent meeting for the subjects to read the consent, ask questions and decide whether or not they are willing to participate in the study. As many as 70 subjects will be enrolled in this study. Participants will receive $150 for the completion of each infusion procedure for a total of $300 upon study completion.

There will be no costs to participants for any of the treatments or testing done as part of this research study. Immediate necessary care is available if an individual is injured because of participation in a research project. However, there is no provision for free medical care or for monetary compensation for such injury. During this study, hospitalizations or additional care beyond the scope of this study will be the responsibility of patients and/or their insurance company.

RISK/BENEFIT ASSESSMENT: This study provides no potential benefit to the participant. The following list describes potential risks associated with this protocol:

- The potential risks from IV placement include momentary discomfort, bleeding, bruising, inflammation, and rarely infection or fainting. A nurse will be present during the infusion procedures to monitor the IV access points and subjects will be asked to alert the study nurse if they experience any pain at the catheter site.

- The risks from infusion of micromolar amounts of Exendin-9 are remote. The IND has been obtained from the FDA for the infusion of Exendin-9 [#65837] into humans. The Principal Investigator for the present study has used this peptide in several previous studies, in doses similar to those described in this application, with no attributable adverse effects observed. Allergic reactions to synthetic peptides are possible; therefore skin testing will be performed on each subject to assess sensitivity prior to undergoing any clamp procedures. All peptides will be synthesized to GMP standards and documented sterile and pyrogen free. Compounding of the peptide will be performed by the Duke Investigational Drug Service (IDS).

- The amount of blood to be withdrawn during any one of these infusion procedures is approximately 159ml and does not constitute an undue risk in persons with normal hematocrits.

- There is a minor risk of hypoglycemia associated with the drug Sitagliptin for the non-diabetic cohort. We will monitor glucose every 15 minutes using an YSI analyzer to ensure that glucose levels remain within a safe range for the participants.

- Sitagliptin is a medication approved by the FDA for the treatment of type 2 diabetes mellitus. In this study, Sitagliptin will not be used for treatment of diabetes but will instead be used to study how the molecule GLP-1 functions in diabetic and non-diabetic individuals. Adverse effects have been reported in studies of individuals taking Sitagliptin. These include:
    - Less likely (approximately 5% of individuals):
        - Low blood sugar (hypoglycemia)
        - Upper respiratory infection
        - Headache
        - Nasal / throat infection
    - Rare but serious events
        - Severe allergic reaction
        - Inflammation of the pancreas

  - Loss of normal kidney function
  - joint pains
- Arginine is a simple amino acid that is produced in the body. The injection of a bolus of arginine over one minute may cause symptoms such as nausea or a strange taste in the mouth.

- At study entry, women of child-bearing potential will have a serum pregnancy test. The pregnancy test must be negative before the subject can undergo any study testing.

SUBJECT COMPETENCY: Subjects who have diminished capacity or who do not have the capacity to give legally effective consent will not be included. This assessment will be made by the study coordinator at the initial consent meeting.

DATA ANALYSIS AND STATISTICAL CONSIDERATIONS: The primary outcome will be arginine-stimulated insulin secretion with and without GLP-1r blockade. We will use the mean increment of insulin secretion rate (ISR) above baseline in the 15 minutes after arginine stimulation to integrate insulin secretion, the primary outcome measure, and two-way analysis of variance with sitagliptin/no mediation, and Ex-9/saline as the two factors; post-hoc comparisons among the 4 insulin responses will be made if a significant interaction is present. In our previous studies, mean insulin concentrations in healthy subjects during an arginine bolus were 104 ± 14 uU/ml, with a coefficient of variance (CV) of 49%. Using this estimate of between subject variation, to detect a 25% difference between the Ex-9 and control groups with a power of 80% at a significance level of 0.05 will require 18 subjects. We plan to finish 20 subjects for each of the study cohorts.

DATA STORAGE AND CONFIDENTIALITY: All study documents will be stored in a locked office to be accessed only by key personnel on the study. Any electronic data will be stored on the study coordinator's password protected office computer, which is used only by the coordinator. Study records that identify subjects will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, they will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS). For records disclosed outside of DUHS, they will be assigned a unique code number. The key to the code will be kept in a password-protected electronic file accessible only to the PI and study coordinator.

DATA SAFETY MONITORING PLAN: As the PI has performed similar studies with humans in the past, a Data Safety and Monitoring Plan (DSMP) has already been established for studies involving synthetic peptides such as exendin-9. This DSMP will continue for the oversight of this project.

No toxicities of Exendin-9 have been described in previous studies in humans. For the purposes of this study, a serious adverse event is defined as any of the following: death, life threatening event, or an event resulting in permanent disability, hospitalization, or prolongation of a hospital stay. Thus, the intervention and protocol represents slightly more than minimal risk to the research subjects. Due to the low risk status of this study, the data safety monitoring plan (DSMP) for this project represents the monitoring for adverse events by the principal investigator (PI) in conjunction with a Safety Officer.

The Safety Officer for this trial will be William E. Kraus, MD. Dr. Kraus is a practicing Cardiologist who has experience in trial design and clinical trials involving diabetes and carbohydrate metabolism. He is familiar with the procedures used in this project and is very familiar with the mechanisms for reporting adverse events. As Safety Officer, Dr. Kraus will review the reports sent to him by the PI or his designees and will determine whether

corrective action should be communicated to the PI, the IRB, the GCRC, NIH, and the FDA. The Safety Officer will be available to discuss glycemic control strategies with respect to the individual subject's medical care goals and the goals necessary for the study. If the goals and strategies necessary for the study exceed those necessary for medical care or practical for that individual, the PI and/or Safety Officer may choose to exclude the subject or discharge them from the study. Annually or at the completion of an Aim, the Safety Officer will complete a checklist that is used as a reminder regarding the functions he is to perform. We will prospectively collect data regarding potential adverse events during the course of the study and collate them on an annual basis. Since the PI and study team at the Center for Living will have frequent contact with the subjects, they are ideally suited to document the presence of Adverse Events.

There are a number of expected adverse events which will be documented on the case report form but will not be documented as serious adverse events. These include mild hypoglycemic reactions, transient nausea and/or dizziness, and bruising/phlebitis at IV sites as these do not qualify as serious adverse events in this project.

In this protocol, it is very unlikely that excess adverse events would occur that would require stopping the study. As outlined elsewhere, adverse events will be monitored and reviewed by the Safety Officer who would make any determination regarding stopping the protocol because of unacceptable risks to subjects. In addition, it is very unlikely that new information would become available during the study that would affect the protocol's integrity. This information would be captured on the Safety Officer Checklist which is reviewed annually or at the completion of an Aim.

In accordance with federal regulations the PI will monitor for, review, and promptly report to the IRB, appropriate institutional officials, sponsor, coordinating center and the appropriate regulatory agency head all unanticipated problems involving risks to subjects or others that occur in the course of a subject's participation in a research study (45 CFR 46.103(b)(5)(i) and 21 CFR 56.108(b)(1)), and all reportable AEs will be submitted per the DUHS IRB policies; available at: (http://irb.duhs.duke.edu/modules/irb_pols/index.php?id=2)

Note Regarding Change in Drug Preparation: Due to unexpected low levels of peptide present in the prepared drug solutions, we are submitting an update to the drug preparation process for this protocol. Moving forward, we plan to dissolve the GLP-1 (32977), Ex-9 (65837), and GIP (110035) peptides using human serum albumin to stabilize the drug preparation and delivery. This is the same approach we've used in previous protocols filed under these same INDs and would like to continue using that approach. This change in drug preparation does not affect subject risk, however a statement has been added to the informed consent form to notify subjects that trace amounts of human serum albumin will be used during the drug preparation in case individuals would elect to opt out of the study due to the use of human blood product.