Study Title: Regulation of Insulin Secretion by the GLP-1 Receptor

Principal Investigator: David D'Alessio

NCT02844907

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DUHS IRB Application (Version 1.5)

General Information

*Please enter the full title of your protocol:		
Regulation of Insulin Secretion by the GLP-1 Receptor		
*Please enter the Short Title you would like to use to reference the study:		
Paracrine Effects on Insulin Secretion * This field allows you to enter an abbreviated version of the Study Title to quickly identify this study.		

Research Abstract

Please type your Research Abstract here:

The Research Abstract should summarize the main points of your study in one paragraph. The following guidelines may help you:

1. Purpose and objective (1-2 sentences)
2. Study activities and population group (2-4 sentences)
3. Data analysis and risk/safety issues (1-2 sentences)

This protocol is funded by the Veteran's Administration and being led by Dr. David D'Alessio in the Division of Endocrinology and the Duke Molecular Physiology Institute.

PURPOSE OF STUDY:

1. Determine the interaction of GLP-1 and GIP to regulate insulin secretion in nondiabetic subjects. The first part of this aim will determine the threshold plasma concentration of GLP-1 that increases insulin secretion to test the hypothesis that fasting levels of circulating GLP-1 are insufficient to mediate β-cell secretion. The second part of the aim will determine whether the insulin response to specific amino acids is dependent on the α-cell release of GLP-1.
2. Determine the role of basal GLP-1 action on the β-cell response to insulin resistance. Healthy subjects will have fasting GLP-1 action determined with GLP-1r blockade before and after induction of experimental insulin resistance. We hypothesize that fasting GLP-1 action will increase to compensate for experimental insulin resistance.
3. Determine the role of basal GLP-1 action on fasting glucose regulation in lean, obese, pre-diabetic and diabetic subjects. A cross sectional study of age-matched subjects across the spectrum of glucose tolerance will be used to test the hypothesis that fasting GLP-1 action increases as β-cell function declines. Groups of lean, obese, pre-diabetic and type 2 diabetic (T2DM) subjects will receive exedin-9 during a graded glucose infusion. We hypothesize that the relative effect of GLP-1r blockade on insulin secretion will be greater with progression from normal to T2DM.

The initial protocol submitted and approved by the Duke IRB was intended to focus solely on the design and procedures for Aim 1 in order to address the three aims listed below in sequential order such that the findings from Aim 1 would be used to further develop the protocol design and procedures for Aim 2, and similarly the findings from Aims 1 and 2 will strengthen the approach to Aim 3.

This study will enroll up to 40-60 total participants for Aim-2 and Aim-3. While we anticipate that the screening blood draw will yield a number of screen failures, we expect that approximately 20-25 subjects will complete Aim-2 and 15-20 will complete Aim-3 of the study protocol.

Enrolled participants for Aim-2 will be asked to complete three study visits including the consent visit and two infusion study visits that will include hyperglycemic clamps and an infusion of Exedin-9. Each

infusion procedure will last 2 hours.

AIM-2: The primary outcome for visits 2-3 will be to measure the effects of Ex-9 on fasting glucose-stimulated insulin secretion before and after experimentally induced insulin resistance. The primary experimental variable for analysis will be C-peptide during the clamp. Mean values will be compared between the period of glucose only stimulation and glucose with Ex-9. For each subject in the active treatment arm data will be analyzed using 2-way ANOVA for repeated measures using time (0-60 vs 60-120 min) and treatment (Dex vs no Dex) as the two factors. Based on our previous studies we expect a significant time effect due to Ex-9. If there is an interaction with treatment we would conclude that experimental insulin resistance influences the fasting GLP-1 effect. Data from control subjects will be analyzed identically; here we expect no interaction, indicating that the fasting GLP-1 is a stable measure. In our previous study using Ex-9 during a glucose clamp, the average coefficient of variation in insulin concentrations at the conclusion of each step in the ramp was 30%. Using this estimate of between subject variation, detecting a 20% difference between subsequent steps in the GLP-1 ramp with a power of 80% and significance level of 0.05 will require 8 subjects.

Enrolled participants for Aim-3 will be asked to complete two study visits including the consent visit and one infusion study visit that will include a hyperglycemic clamp and an infusion of Exedin-9. The infusion procedure will last 2 hours.

AIM-3 – The primary outcome measure for this study will be the fasting GLP-1 effect. This will be defined as the difference in steady state glucose-stimulated insulin secretion with and without Ex-9. The difference will be expressed as a percentage of glucose stimulated insulin secretion without Ex-9, and serve as the primary variable for comparison among the lean, obese and diabetes cohorts.

Research Summary

State your primary study objectives

AIM-2: Determine the role of basal GLP-1 action on the β-cell response to insulin resistance. Healthy subjects will have fasting GLP-1 action determined with GLP-1r blockade before and after induction of experimental insulin resistance. We hypothesize that fasting GLP-1 action will increase to compensate for experimental insulin resistance.

AIM-3: The primary outcome measure for this study will be the fasting GLP-1 effect. This will be defined as the difference in steady state glucose-stimulated insulin secretion with and without Ex-9.

State your secondary study objectives

Please select your research summary form:

Standard Research Summary Template

This is the regular (generic) research summary template which is required for all regular applications (unless your protocol fits under the other research summary templates in this category). Use of these instructions is helpful for ensuring that the research summary contains all necessary elements.

Standard Research Summary

Purpose of the Study

- Objectives & hypotheses to be tested

This protocol is funded by the Veteran's Administration and being led by Dr. David D'Alessio in the Division of Endocrinology and the Duke Molecular Physiology Institute. The study will address the following three specific aims:

1. **Determine the roles of fasting GLP-1 concentrations and alpha-cell secretion on insulin release.** The first part of this aim will determine the threshold plasma concentration of GLP-1 that increases insulin secretion to test the hypothesis that fasting levels of circulating GLP-1 are insufficient to mediate β-cell secretion. The second part of the aim will determine whether the insulin response to specific amino acids is dependent on the α-cell release of GLP-1.
2. **Determine the role of basal GLP-1 action on the β-cell response to insulin resistance.** Heal thy subjects will have fasting GLP-1 action determined with GLP-1r blockade before and after induction of experimental insulin resistance. We hypothesize that fasting GLP-1 action will increase to compensate for experimental insulin resistance.
3. **Determine the role of basal GLP-1 action on fasting glucose regulation in lean, obese, pre-diabetic and diabetic subjects.** A cross sectional study of age-matched subjects across the spectrum of glucose tolerance will be used to test the hypothesis that fasting GLP-1 action increases as β-cell function declines. Groups of lean, obese, pre-diabetic and type 2 diabetic (T2DM) subjects will receive exedin-9 during a graded glucose infusion. We hypothesize that the relative effect of GLP-1r blockade on insulin secretion will be greater with progression from normal to T2DM.

Initially, this study was to address these three aims in sequential order such that the findings from Aim 1 would be used to further develop the protocol design and procedures for Aim 2, and similarly the findings from Aims 1 and 2 will strengthen the approach to Aim 3. To that end, Dr. D'Alessio has been able to address Aim 1 by collecting and analyzing data from another study with a similar protocol design. This IRB amendment is being filed to address the procedures for both Aim 2 and Aim 3.

Background & Significance

- Should support the scientific aims of the research

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 b-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 a-cells acts in a paracrine manner to regulate neighboring b-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 b-cell demand. Taken together, the published literature raises the possibility that intra-islet GLP-1 action is a mechanism of b-cell compensation. If true, this model would dramatically shift understanding of the incretin system and present a range of new opportunities for diabetes treatment.

The GLP-1r signaling system has been the centerpiece of diabetes drug development in the past decade with two classes of drugs, peptide agonists with extended activity in circulation and DPP-4 inhibitors that prolong the action of endogenous GLP-1. The effectiveness of these agents attests to the importance of GLP-1 signaling in glucose regulation. The central hypothesis guiding this grant proposal is that fasting GLP-1 action, mediated by paracrine effects of α-cell GLP-1, contributes to the control of insulin secretion and the β-cell compensation for insulin resistance and glucose intolerance. Testing this hypothesis is important for clarifying the mechanism of action of a vital pathway regulating β-cells and for refining the use of GLP-1 signaling for the treatment of diabetes and pre-diabetes.

Design & Procedures

- Describe the study, providing detail regarding the study intervention (drug, device, physical procedures, manipulation of the subject or the subject's environment, etc.). Discuss justifications for placebo control, discontinuation or delay of standard therapies, and washout periods if applicable. Identify procedures, tests and interventions performed exclusively for research purposes or more

frequently than standard of care. Include alternative therapies, concurrent therapies discontinued per protocol, risk benefit ratio, and use of tissue/specimens. Discuss monitoring during washout periods if applicable. Include brief description of follow-up, if any.

The entire study (Aims 1-3) will enroll up to 60 participants. While we anticipate that the screening blood draw will yield a number of screen failures, we expect that approximately 20-25 subjects will complete AIM-2 and 15-20 will complete AIM-3 of the 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.

AIM-2

Approximately 20-25 healthy subjects will complete Aim-2 of the study and these participants will have three study visits – a baseline consent visit, and two infusion procedures.

Visit 1: The consent visit will take place at the Duke Center for Living and will last approximately two hours. If the participant has given prior verbal consent during the phone screening process, they will fast (nothing but water) for 12 hours before this visit and their body mass index (composed of weight and height measures) will be checked to see if it falls within the inclusion range. Daily medications may be taken. 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 hemoglobin A1c (HbA1c), fasting glucose level, kidney function (i.e. eGFR) and blood count (i.e. hematocrit) are within normal limits 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.

If a woman is of childbearing potential, a blood (using one teaspoon of blood drawn by a needle-stick) pregnancy test will be conducted and it must be negative prior to continuation in this study.

Visits 2 and 3: Each participant will have two 4-hour hyperglycemic clamp procedures with the addition of the GLP-1 receptor antagonist Exendin-9 (Ex-9) to determine the *fasting GLP-1 effect*. During both visits, the participants will receive an infusion of Ex-9 beginning at the 60-minute mark of the clamp procedure; this will allow a comparison of glucose-stimulated insulin secretion with and without GLP-1 action, a parameter we have termed the *fasting GLP-1 effect*. Throughout the glucose infusions during visits 2 and 3, 1 ml blood samples will be taken every 5-10 minutes beginning at the -15 minute time-point and continue until the end of the 120 minute procedure. These samples will be tested immediately with a YSI glucose analyzer and the values used to adjust the glucose infusion rate to maintain stable blood glucose concentrations (a glucose clamp) throughout the experiment.

Subjects will report to the Duke Center for Living after a 12-hour fast. Study staff will obtain the participant's current body weight. At Visit-2 a skin test to confirm they do not have any sensitivity to the Ex-9 investigational compound that will be used during both visits 2 and 3 will be administered. 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. This skin test must be negative in order to continue with the study testing. Once a negative skin test has been recorded, the participant will not need to be retested for sensitivity to Ex-9 at visit 3.

Subjects will have two intravenous catheters placed, one in each arm: 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. Four (4.0 ml) fasting blood samples will be taken at -15, -10, -5 and 0 minutes and then a variable infusion of 20% dextrose will begin to clamp the blood glucose at basal +3 mM for 120 minutes. This hyperglycemic clamp will be stabilized over 60 minutes and 4.0 ml blood samples will be taken every 5-10 minutes during this period to establish baseline insulin secretion and active GLP-1 levels. During these visits, participants will receive an infusion of Exendin-9 (750 pmol/kg/min) between the 60 and 120 minute time-points of the clamp procedure. For consistency, blood draws (4.0 ml) will occur every 5-10 minutes between the 60 and 120 minute time-points of both visit 2 and 3. Upon completion of the final 60-minute interval for the Ex-9 infusion, both the infusion and clamp procedures will be complete. One additional blood sample (1 ml) will be drawn post the assessment to be tested immediately with the YSI glucose analyzer to ensure glucose levels are within normal limits. **Visit 3** will occur at least one week later and will be a repeat infusion procedure identical to **Visit 2.** The total amount of blood collected during each of these visits will be a maximum of ~141 ml (~9.5 Tbsp).

We will aim to complete studies in 10-15 subjects before and after approximately one week of dexamethasone(Dex) treatment to induce insulin resistance. Insulin resistance in response to a short-course (4 mg daily) of supraphysiologic glucocorticoids is an established research procedure and is completely reversible over the course of exposure we are proposing. However, the rate of reversal is variable among subjects preventing a straight counter-balanced allocation to treatment (i.e. half of subjects getting Dex before the first study and half before the second). Therefore we will enroll 6-10 subjects as controls for effects of time and repeat testing. These individuals will have identical clamps with no dexamethasone at approximately 1 week intervals and will be determined at random by study staff.

AIM-3

Approximately 15-20 subjects will complete AIM-3 of the study and these participants will have two study visits – a baseline consent visit, and one infusion procedures.

Visit 1: Visit 1 is a baseline study visit during which the following will take place:
• Potential subjects will review and sign the consent form with study staff.
• The participant's current medications will be recorded for review by study staff.
• A screening blood draw will be obtained to measure fasting glucose, HbA1c, hematocrit, and eGFR.

If a woman is of childbearing potential, a blood (using one teaspoon of blood drawn by a needle-stick) pregnancy test will be conducted and it must be negative prior to continuation in this study.

Visit 2: Similar to Aim 2, each participant will have a 4-hour hyperglycemic clamp procedure with the addition of the GLP-1 receptor antagonist Exendin-9 (Ex-9) to determine the *fasting GLP-1 effect*. The participants will receive an infusion of Ex-9 beginning at the 60-minute mark of the clamp procedure. Throughout the glucose infusion during visit-2, 1 ml blood samples will be taken every 5-10 minutes beginning at the -15 minute time-point and continue until the end of the 120 minute procedure. These samples will be tested immediately with a YSI glucose analyzer and the values used to adjust the glucose infusion rate to maintain stable blood glucose concentrations (a glucose clamp) throughout the experiment.

Subjects will report to the Duke Center for Living after a 12-hour fast. Study staff will obtain the participant's current body weight. At Visit-2 a skin test to confirm they do not have any sensitivity to the Ex-9 investigational compound that will be used during the infusion will be administered. 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. This skin test must be negative in order to continue with the study testing.

Subjects will have two intravenous catheters placed, one in each arm: 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. Four (4.0 ml) fasting blood samples will be taken at -15, -10, -5 and 0 minutes and then a variable infusion of 20% dextrose will begin to clamp the blood glucose at basal +3 mM for 120 minutes. This hyperglycemic clamp will be stabilized over 60 minutes and 4.0 ml blood samples will be taken every 5-10 minutes during this period to establish baseline insulin secretion and active GLP-1 levels. During these visits, participants will receive an infusion of Exendin-9 (750 pmol/kg/min) between the 60 and 120 minute time-points of the clamp procedure. For consistency, blood draws (4.0 ml) will occur every 5-10 minutes between the 60 and 120 minute time-points. Upon completion of the final 60-minute interval for the Ex-9 infusion, both the infusion and clamp procedures will be complete. One additional blood sample (1 ml) will be drawn post the assessment to be tested immediately with the YSI glucose analyzer to ensure glucose levels are within normal limits. The total amount of blood collected during this visit will be a maximum of ~141 ml (~9.5 Tbsp).

Selection of Subjects

- List inclusion/exclusion criteria and how subjects will be identified.

AIM-2 SELECTION OF HEALTHY SUBJECTS: Study recruitment will occur primarily through advertisements via Duke websites, as well as local newspapers (e.g. Independent, Durham Herald, Duke Chronicle); and social media venues (e.g. DMPI Facebook page, Instagram), will be used as well as use of our Duke Health and Exercise Research Trials registry. Study recruitment will also take place at public venues (e.g. Duke Farmer's Market or Community Health Fairs). Potential subjects for this study will be directed to contact the study staff to obtain more information about the study, if interested. Study participants will also be recruited using flyers posted across the Durham VAMC hospital and Duke hospital /clinic facilities, inviting interested individuals to contact the study recruitment coordinator for additional information.

Inclusion criteria:

- Healthy adults age 20-45 years
- Male or female
- Body Mass Index (BMI) ≤ 35.0 kg/m^2
- HbA1c ≤ 5.7%

- Ability to speak and understand English

Exclusion criteria:

- Uncontrolled high blood pressure
- Diabetes or use of diabetes medications such as insulin or metformin
- Evidence of active heart disease or heart failure
- Lung disease or COPD
- Malabsorptive GI disease, such as celiac disease, or gastric bypass
- Significant hepatic disease
- Kidney disease or renal insufficiency (eGFR < 60 mL/kg/min)
- Untreated anemia (hematocrit < 34%) as measured at screening visit
- Pregnant females
- Active substance abuse
- Chronic use of oral steroid medications such as prednisone and hydrocortisone
- Apparent sensitivity to any of the study peptides as determined by the skin test
- Diagnosis or h/o PTSD
- Active mental health disorders such as depression, or as a result of Traumatic Brain Injury (TBI)

Exclusion criteria will primarily be identified by way of a phone screen process conducted by the 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.

AIM-3 SELECTION OF SUBJECTS:

Selection of Non-Diabetic Subjects: Study recruitment will occur primarily through advertisements via Duke websites, as well as local newspapers (e.g. Independent, Durham Herald, Duke Chronicle); and social media venues (e.g. DMPI Facebook page, Instagram) will be used as well as use of our Duke Health and Exercise Research Trials registry. Study recruitment will also take place at public venues (e.g. Duke Farmer's Market or Community Health Fairs). Potential subjects for this study will be directed to contact the study staff to obtain more information about the study, if interested.

Study participants will also be recruited using flyers posted across the Durham VAMC hospital and Duke hospital/clinic facilities, inviting interested individuals to contact the study recruitment coordinator for more information. In addition, the VA Informatics and Computing Infrastructure (VINCI) will be used to identify potential subjects that meet the inclusion criteria. The study staff will pre-screen subjects listed on the VINCI reports in CPRS and contact those subjects identified to meet the inclusion criteria. Recruitment may occur through the VA Endocrinology practice and primary care clinics.

Inclusion criteria - Non-Diabetic Subjects:

- Adults age 18-65 years
- Male or female
- Body Mass Index (BMI) 25-40 kg/m^2
- HbA1c ≤ 6.5% as measured at screening visit
- No Diabetes or use of diabetes medications such as insulin or metformin
- Ability to speak and understand English

Selection of T2DM Subjects: Methods for subject recruitment will be similar to the non-diabetic cohort with additional recruitment through the VA Endocrinology practice and primary care clinics, as well as potential assistance from search results from Slicer Dicer and our Duke Health and Exercise Research Trials or DCRU subject registries.

Inclusion criteria - T2DM Subjects:

- Adults age 18-65 years
- Male or female
- Body Mass Index (BMI) 25-40 kg/m^2
- HbA1c ≤ 7.5% plus a diagnosis of Type II diabetes managed by either Metformin, Sulfonylurea, or diet and exercise

Exclusion criteria: ALL SUBJECTS

- Uncontrolled high blood pressure
- Evidence of active heart disease, unstable angina or heart failure
- Lung disease or COPD
- Malabsorption or GI disease, such as celiac disease, or gastric bypass
- Significant hepatic disease
- Kidney disease or renal insufficiency (eGFR < 60 mL/kg/min)

- Untreated anemia (hematocrit < 34%) as measured at screening visit
- Pregnant females
- Active substance abuse
- Chronic use of oral steroid medications such as prednisone and hydrocortisone
- Apparent sensitivity to study peptides as determined by the skin test
- Diagnosis or h/o PTSD
- Active mental health disorders such as depression, or as a result of Traumatic Brain Injury (TBI)

Exclusion criteria will primarily be identified by way of a phone screen process conducted by the coordinator /study staff, however medical record review for both cohorts may be used to confirm the absence of other medical conditions listed as exclusion criteria.

Subject Recruitment and Compensation

- Describe recruitment procedures, including who will introduce the study to potential subjects. Describe how you will ensure that subject selection is equitable and all relevant demographic groups have access to study participation (per 45 CFR 46.111(a) (3)). Include information about approximately how many DUHS subjects will be recruited. If subjects are to be compensated, provide specific prorated amounts to be provided for expenses such as travel and/or lost wages, and/or for inducement to participate.

Study recruitment will occur primarily through advertisements via Duke websites, as well as local newspapers (e.g. Independent, Durham Herald, Duke Chronicle); and social media venues (e.g. DMPI Facebook page, Instagram), will be used. Study recruitment will also take place at public venues (e.g. Duke Farmer's Market or Community Health Fairs). Potential subjects for this study will be directed to contact the study staff to obtain more information about the study, if interested. While the study staff will be a Duke employee on a WOC agreement with the Durham VAMC, all phone calls will be made using a VA cell phone and data obtained during the phone screen will immediately be entered into a database on a VA encrypted laptop. When contacted, the study staff will confirm the participant meets most of the inclusion /exclusion criteria before scheduling him/her for a consent meeting. Potential subjects will attend a group study information session that will be conducted by the study coordinator or another member of key personnel. We will allow approximately 30 minutes at the end of the information session for the subjects to read the consent, ask questions in a separate one-on-one setting and decide whether or not they are willing to participate in the study. As many as 60 subjects may be enrolled in this entire study (AIMS 1-3) as we seek to identify 45 individuals (for both AIM-2 and AIM-3 combined) that meet all inclusion/exclusion criteria and complete the study. Participants will receive $125 for the completion of each infusion procedure for a maximum of $250 (AIM-2) or $125 (AIM-3) upon study completion. Participant payments will be made through the Durham VA Medical Center's cashier office. For Veterans with bank accounts, study staff members are required to complete a "Vendorizing Coversheet" with the participant, which includes bank account information. Any participant who does not have a bank account allowing direct deposit will be paid via checks issued through VA Financial Services in Austin, TX and mailed to their address on file. Participants will be informed that payment will be received approximately 4 to 6 weeks after it is requested. For subjects who do not have a bank account or a physical address, payment will be made through the agent cashier.

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.

Subject's Capacity to Give Legally Effective Consent

- If subjects who do not have the capacity to give legally effective consent are included, describe how diminished capacity will be assessed. Will a periodic reassessment occur? If so, when? Will the subject be consented if the decisional capacity improves?

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.

Study Interventions

- If not already presented in #4 above, describe study-related treatment or use of an investigational drug or biologic (with dosages), or device, or use of another form of intervention (i.e., either physical procedures or manipulation of the subject or the subject's environment) for research purposes.

Risk/Benefit Assessment

- Include a thorough description of how risks and discomforts will be minimized (per 45 CFR 46.111(a) (1 and 2)). Consider physical, psychological, legal, economic and social risks as applicable. If vulnerable populations are to be included (such as children, pregnant women, prisoners or cognitively impaired adults), what special precautions will be used to minimize risks to these subjects? Also identify what available alternatives the person has if he/she chooses not to participate in the study. Describe the possible benefits to the subject. What is the importance of the knowledge expected to result from the research?

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-(9-39) are remote. In rare cases, nausea and vomiting have been reported as possible side effects for Ex-9. The IND has been obtained from the FDA for the infusion of exendin-(9-39) [#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.
- The maximum amount of blood to be withdrawn during any one of these infusion procedures is approximately 141 ml and does not constitute an undue risk in persons with normal hematocrits.
- 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.
- AIM-2: Some side effects of dexamethasone may include upset stomach, headache, dizziness, insomnia, restlessness, increased appetite or weight gain may occur.

This is not a complete list of possible side effects. If the participant notices other effects not listed above, they will be advised to contact the study physician.

Costs to the Subject

- Describe and justify any costs that the subject will incur as a result of participation; ordinarily, subjects should not be expected to pay for research without receiving direct benefit.

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.

Data Analysis & Statistical Considerations

- Describe endpoints and power calculations. Provide a detailed description of how study data will be analyzed, including statistical methods used, and how ineligible subjects will be handled and which subjects will be included for analysis. Include planned sample size justification. Provide estimated time to target accrual and accrual rate. Describe interim analysis including plans to stop accrual during monitoring. Phase I studies, include dose escalation schema and criteria for dose escalation with definition of MTD and DLT.

AIM-2 – The primary outcome for visits 2-3 will be to measure the effects of Ex-9 on fasting glucose-stimulated insulin secretion before and after experimentally induced insulin resistance. The primary experimental variable for analysis will be C-peptide during the clamp. Mean values will be compared between the period of glucose only stimulation and glucose with Ex-9. For each subject in the active treatment arm data will be analyzed using 2-way ANOVA for repeated measures using time (0-60 vs 60-120 min) and treatment (Dex vs no Dex) as the two factors. Based on our previous studies we expect a significant time effect due to Ex-9. If there is an interaction with treatment we would conclude that experimental insulin resistance influences the *fasting GLP-1 effect*. Data from control subjects will be analyzed identically; here we expect no interaction, indicating that the fasting GLP-1 is a stable measure. In our previous study using Ex-9 during a glucose clamp, the average coefficient of variation in insulin concentrations at the conclusion of each step in the ramp was 30%. Using this estimate of between subject variation, detecting a 20% difference between subsequent steps in the GLP-1 ramp with a power of 80% and significance level of 0.05 will require 8 subjects.

AIM-3 – The primary outcome measure for this study will be the fasting GLP-1 effect. This will be defined as the difference in steady state glucose-stimulated insulin secretion with and without Ex-9. The difference will be expressed as a percentage of glucose stimulated insulin secretion without Ex-9, and serve as the primary variable for comparison among the lean, obese and diabetes cohorts.

Data & Safety Monitoring

- Summarize safety concerns, and describe the methods to monitor research subjects and their data to ensure their safety, including who will monitor the data, and the frequency of such monitoring. If a data monitoring committee will be used, describe its operation, including stopping rules and frequency of review, and if it is independent of the sponsor (per 45 CFR 46.111(a) (6)).

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-(9-39). This DSMP will continue for the oversight of this project.

No toxicities of exendin-(9-39) or GLP-1 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 not be documented. 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. 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. These will be documented and reviewed by the safety officer as they arise.

In this low risk 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, and all reportable AEs will be submitted per the DUHS IRB policies.

Describe Role of External Personnel:

Not applicable.