COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title:
Can Exenatide Prevent the Increase in EGP in Response to Dapagliflozin-induced Increase in Glucosuria

NCT number: NCT03331289

IRB Approval Date: 03/21/2019

Unique Protocol ID: HSC20170582H

Protocol Template Form

Item 1 UTHSCSA Tracking Number	HSC20170582H

Item 2 Abstract / Project Summary	Provide a succinct and accurate description of the proposed research. State the purpose/aims. Describe concisely the research design and methods for achieving the stated goals. This section should be understandable to all members of the IRB, scientific and non-scientific. **DO NOT EXCEED THE SPACE PROVIDED**.

Purpose/Objectives: To examine whether coadministration of the GLP-1 receptor agonist exenatide (a potent inhibitor of glucagon and stimulator of insulin secretion) with an SGLT2 inhibitor dapagliflozin can prevent/ameliorate the compensatory rise in Endogenous Glucose Production (EGP) and produce an additive, even synergistic decrease in the plasma glucose concentration

Research Design/Plan:After screening, each subject will receive 1 measurements of EGP with prime-continuous Infusion of 3-3H-glucose. After completing the EGP measurement each subject will receive a Double Tracer OGTT.

Methods: Visit 1: Screening. Medical history will be obtained, physical exam performed, and pregnancy test performed.
Visit 2: Endogenous Glucose Production Measurement: The rate of EGP will be measured with 3-3H-glucose.
Visit 3: Double Tracer OGTT

Clinical Relevance: The primary end point is the change in EGP. The difference in rate of EGP during the last hour of the study (from 240-300 minutes) between drug-treatment and placebo treatment studies represents the effect of drug treatment on EGP, which will be compared among the 3 drug. treatments (exenatide; dapagliflozin; exenatide plus dapagliflozin) with ANOVA

Item 3 Background	
Describe past experimental and/or clinical findings leading to the formulation of your study. *For research involving unapproved drugs, describe animal and human studies.* *For research that involves approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.*	Insert background: Glucosuria produced by inhibition of the renal sodium-glucose transporter-2 (SGLT2) lowers the fasting plasma glucose (FPG) concentration but causes a "paradoxical" increase in endogenous glucose production (EGP). Although the increase in EGP can be viewed as a compensatory mechanism that opposes urinary glucose loss and prevents the development of hypoglycemia in NGT individuals, in T2DM individuals it occurs while the plasma glucose concentration is still in the hyperglycemic range. Moreover, it offsets by ~50% the urinary glucose loss produced by SGLT2 inhibitors and attenuates their glucose lowering ability. The increase in EGP following SGLT2 inhibition is associated with an increase in plasma glucagon concentration and decrease in plasma insulin concentration. Because renal glucose production is stated to be unresponsive to an increase in the plasma glucagon concentration, it is likely that the liver contributes, at least in part, to the increase in EGP [which is triggered by glucosuria]. However, an increase in glucose production by the kidney cannot be excluded. We hypothesize that there is a previously unrecognized, [completely novel] "reno-hepatic" interaction that participates in the regulation of plasma glucose concentration. This "reno-hepatic axis" is [activated by glucosuria] and stimulates hepatic, as well as renal, glucose production. The aim of the present study is to: (i) determine the source of increase in EGP, liver versus kidney [or both]; and (ii) examine the signal activated by glucosuria that is responsible for the increase in EGP.

Item 4 Purpose and rationale *Insert purpose, objectives and research questions/hypotheses here.* *If you cut and paste from another document, make sure the excerpted material answers the question*	Insert purpose: Understanding the factors that regulate EGP is key to understanding normal glucose homeostasis and development of hyperglycemia in T2DM. We and others have shown that glucosuria produced by SGLT2 inhibition (SGLT2i) stimulates EGP, indicating the presence of an interaction between the kidney and liver, i.e. "a renohepatic axis" that coordinates the regulation of EGP and plasma glucose conc . The present study will provide evidence for the existence of this previously unrecognized reno-hepatic axis and define the mechanisms responsible for the interaction between the kidney and liver. In addition to providing novel insights about the regulation of glucose homeostasis, the present study has important clinical implications. Although SGLT2i are effective in producing glucosuria, they cause only a modest decrease in HbA1c (0.6-0.8%). On one hand, the presence of a "reno-hepatic axis" that leads to a compensatory increase in EGP in response to glucosuria maintains the FPG in NGT individuals and prevents hypoglycemia. On the other hand, the increase in EGP in T2DM individuals is "paradoxical" in that it occurs while plasma glucose conc is in the hyperglycemic range and offsets by ~50% urinary glucose loss (10) (see results below) produced by SGLT2 inhibitors and attenuates their glucose lowering effect. Thus, determining the mechanisms that mediate the rise in EGP in response to glucosuria will allow the development of strategies that prevent the rise in EGP and increase the efficacy of SGLT2.

Item 5 Study Population(s) Being Recruited In your recruitment plan, how many different populations of prospective subjects do you plan to target? Provide number: 1 *e.g., a population can be individuals with type 2 diabetes controlled with diet and/or a population of healthy controls. Or a population can be individuals attending an education program, etc.* <u>List each different population</u> on a separate row and provide a short descriptive **label**: *(e.g., normal-healthy, diabetics, parents, children, etc.)* *To add rows use copy & paste*	Identify the criteria for **inclusion**:	Identify the criteria for **exclusion**:
T2DM according to ADA criteria	subjects must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, EKG and urinalysis 18-70 years old 21-45 kg/m Male and Female ≥7.0% and≤ up to 10.5% Drug naïve and/or on a stable dose (more than 3 months) of metformin and/or sulfonylurea	Subjects who are Type 1 Diabetes, have proliferative diabetic retinopathy, plasma creatinine >1.4 females or >1.5 males will be excluded. Subjects taking drugs known to affect glucose metabolism (other than metformin and sulfonylurea)

Item 6
Research Plan / Description of the Research Methods ***a.*** *Provide a* ***comprehensive narrative*** *describing the* ***research methods****.*
Provide the ***plan for data analysis*** *(include as applicable the* ***sample size calculation).***

Step-by-Step Methods:
After screening, eligible subjects will receive a measurement of endogenous glucose production (EGP) with a prime-continuous infusion of 3-3H-glucose. The EGP measurement will be performed in the morning after a 10-12 hour overnight fast and will last 8.5 hours (from 6 AM to 2:30 PM). After a 3.5-hour tracer equilibration period, subjects (20 per group) will receive one of the following medications: (i) placebo; (ii) exenatide 5 ug subcutaneously; (iii) dapagliflozin (10 mg); and (iv) dapagliflozin 10 mg plus exenatide 5 ug (see flowsheet for EGP). Following the test medication at 9:30 AM, blood samples will be drawn every 15 minutes for an additional 5 hours and plasma glucose, insulin, C-peptide, glucagon, and glucose specific activity will be measured.

Visit 1: Screening. Medical history will be obtained and physical exam will be performed. Blood will be drawn for FPG, routine blood chemistries, CBC, lipid profile, HbA1c, and thyroid function. Urinalysis, EKG, albumin/creatinine ratio and pregnancy test will be performed.

Visit 2: Endogenous Glucose Production Measurement: The rate of endogenous glucose production will be measured with 3-^{3}H-glucose infusion. [3-^{3}H]-glucose infusion will be started at 6 AM and continued until 2:30 PM (5 hours after drug administration). At 6 AM a catheter will be placed into an anticubital vein and a prime (40 uCi x FPG/100)-continuous (0.4 uCi) infusion of [3-^{3}H]- glucose will be started and continued until 2:30 PM. (5 hours after drug administration). Participant's hand will be placed in a box heated to 50-60°C (122-140°F). Baseline blood samples will be obtained at-210, -60, -50, -45, -40, -35, -30, -20, -10, and 0. After 3.5 hours of tracer equilibration blood samples will be obtained every 10-20 minutes from 9:30 AM to 2:30 PM. Plasma glucose, insulin, C-peptide, glucagon, and [3-^{3}H]-glucose specific activity will be measured. Urine will be collected from 6 to 9:30 AM and from 9:30 AM to 2:30 PM. Urinary volume and glucose concentration will be measured and urinary glucose excretion rate calculated. The study will end at 2:30 PM. The total amount of blood taken during this time is 164 ml or 12 tablespoons.

Visit 3: Double Tracer Oral Glucose Tolerance Test (DT-OGTT): Within one to two weeks after the measurement of EGP, all subjects will have a 5-hour DT-OGTT with measurement of plasma glucose, insulin (I), C-peptide (CP), and glucagon concentrations at -180, -6, -5, -45, -40, -35, -30, -20, -10, 0 and every 15-30 minutes thereafter to obtain a measure of overall glucose tolerance, insulin secretion (□CP0-120/□G0-120), insulin sensitivity (Matsuda index [MI]), beta cell function, (□CP0-120/□G0-120 x MI), and suppression of plasma glucagon concentration (64). At 7 AM a catheter will be placed into an anticubital vein and a prime (25 uCi x FPG/100)- continuous (0.25 uCi) infusion of [3-^{3}H]-glucose will be started and continued until 3 PM. Urinary volume and glucose concentration will be measured and urinary glucose excretion rate calculated. The total amount of blood taken during the OGTT is 226 ml about 15 tablespoons. HbA1c will be measured twice, once on the day of the DT-OGTT and once on the day of the EGP measurement.

Note: The repeated double tracer listed in the grant application will not be performed as part of this protocol. The grant will be amended to remove at continuing review of the grant application.

Data Analysis Plan: The primary end point is the change in EGP. The difference in rate of EGP during the last hour of the study (from 240-300 minutes) between drug-treatment and placebo treatment studies represents the effect of drug treatment on EGP, which will be compared among the 3 drug treatments (exenatide; dapagliflozin; exenatide plus dapagliflozin) with ANOVA. Post hoc testing will be performed with Bonferroni correction for multiple comparisons. The following primary comparison will be performed: (i) change in EGP above baseline following dapagliflozin alone versus dapagliflozin/exenatide.

Item 7 Risks Section:

Complete the following table to describe the risks of all **research procedures** listed in Step 2, Institutional Form (items 28-34). *Do not list risks of Routine care procedures here.*

☒ N/A, Risks are described in the informed consent document – do not complete this table.

Research procedures *example:* • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	**Risks** List the reasonably expected risks under the following categories as appropriate:
Chart Review	Serious and likely; ○ Insert risk here or enter "none" Serious and less likely; ○ Insert risk here or enter "none" Serious and rare; ○ Insert risk here or enter "none" Not serious and likely; ○ Insert risk here or enter "none" Not serious and less likely ○ Breach of confidentiality