COVER PAGE FOR PROTOCOL AND STATISTICAL ALNALYSIS

Official Study Title: SGLT2 INHIBITION AND STIMULATION OF ENDOGENOUS GLUCOSE PRODUCTION Significance - Protocol 3: Role of the Renal Nerves in the Increase in EGP in Response to Glucosuria

NCT number: NCT03168295

IRB Approval Date: 01/05/2017

Unique Protocol ID: HSC20160042

Protocol Template Form

Item 1 UTHSCSA Tracking Number	HSC20160042

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: Examining the effect of SGLT2 inhibition on EGP and plasma glucose concentration in diabetic subjects after kidney transplantation (i.e. renal denervation) or in subjects after renal sympathectomy (63) can add insight about the possible role of a neural arc which mediates the changes in plasma glucagon and/or insulin concentration in response to glucosuria.

Research Design/Plan: After screening, eligible subjects will receive 2 measurements of endogenous glucose production with a prime-continuous infusion of 3-3H-glucose. Each measurement will be performed on a separate day in random order after a 10-12 hour overnight fast and will last 8 hours (from 6 AM to 2 PM). After a 3-hour tracer equilibration period, each subject will receive one of the following medications in random order: (i) placebo and (ii) dapagliflozin 10 mg. Following the test medication at 9 AM, blood samples will be drawn every 20 minutes for an additional 5 hours and plasma glucose, insulin, C-peptide, glucagon, catecholamine concentrations and tritiated glucose sp act will be measured.

Methods: Visit 1: Screening
Visit 2: Endogenous Glucose Production Measurement (EGP)
Visit 3: After completing the first EGP measurement, subjects will return to the Diabetes Research Unit for the second study.

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.*	The rise in EGP following SGLT2 inhibition is associated with an increase in plasma glucagon concentration and progressive decline in plasma insulin concentration (10,11). Although SGLT2 transporters have been demonstrated on alpha cells (55), there are no SGLT2 transporters on beta cells (55). Moreover, the rise in plasma glucagon does not precede, but occurs simultaneously with or slightly after the rise in EGP. These observations suggest that glucosuria initiates a neural arc in which activation of the renal sympathetic nerves sends a signal to the brain which in turn leads to: (i) inhibition of insulin secretion; (ii) stimulation of glucagon secretion; (iii) inhibition of hepatic glucose production; or (iv) some combination of the three.
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: Examining the effect of SGLT2 inhibition on EGP and plasma glucose concentration in diabetic subjects after kidney transplantation (i.e. renal denervation) or in subjects after renal sympathectomy (63) can add insight about the possible role of a neural arc which mediates the changes in plasma glucagon and/or insulin concentration in response to glucosuria.

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: *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**:
20 post-renal transplant T2DM subjects	• age = 18-70 years • BMI = 18.5-40 kg/m2 • HbA1c ≥ 7.0% and ≤10.0%. • males or females • Must be at least 3 months post renal transplantation and be on a stable dose of prednisone (≤5 mg/day), tacrolimus, and mycophenolate mofetil • Not taking any antidiabetic medications or who are treated with metformin, sulfonylurea, DPP4 inhibitor, thiazolidinedione or some combination • Must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, T4, EKG and urinanalysis	• Subjects who are taking insulin or SGLT2 inhibitor are excluded • Only subjects whose body weight has not been stable (± 3 lbs) over the preceding three months and/or who participate in an excessively heavy exercise program will be excluded. • Individuals with evidence of proliferative diabetic retinopathy, plasma creatinine >1.4 females or >1.5 males (and eGFR <45ml/min.1.73m2), or 24-hour urine albumin excretion > 300 mg will be excluded.
20 T2DM subjects without renal transplant (CONTROL)	• age = 18-70 years • BMI = 18.5-40 kg/m2 • HbA1c ≥ 7.0% and≤ 10.0%. • males or females • On stable dose (more than 3 months) of monotherapy or combination therapy with metformin and/or a sulfonylurea • Must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, T4, EKG and urinanalysis	• Subjects taking drugs known to affect glucose metabolism (other than metformin and sulfonylurea) • Only subjects whose body weight has not been stable (± 3 lbs) over the preceding three months and/or who participate in an excessively heavy exercise program will be excluded. • Individuals with evidence of proliferative diabetic retinopathy, plasma creatinine >1.4 females or

	•	>1.5 males (and eGFR <45 ml/min.1.73m2), or 24-hour urine albumin excretion > 300 mg will be excluded.

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: Visit 1: Screening. Medical history will be obtained and physical exam performed. Blood will be drawn for FPG, routine blood chemistries, CBC lipid profile, HbA1c, and thyroid function. Urinanalysis, EKG, albumin/creatinine ratio and pregnancy test will be performed. Visits 2 & 3: Endogenous Glucose Production Measurement: The rate of EGP will be measured with 3-3H-glucose infusion. [3-3H]-glucose infusion will be started at 6 AM and continued until 2 PM (5 hours after drug administration). After 3 hours of tracer equilibration, baseline blood samples will be drawn and subjects will receive placebo or dapagliflozin and arterialized blood samples will be obtained every 10-20 minutes from 9 AM to 2 PM. Plasma glucose, insulin, C-peptide, glucagon, catecholamine concentrations and [3-3H]-glucose specific activity will be measured. Blood will also be drawn to monitor kidney function. Urine will be collected from 6 to 9 AM and from 9 AM to 2 PM. Urinary volume and glucose concentration will be measured and urinary glucose excretion rate calculated. The study will end at 2 PM. After completing the first study, subjects will return to the unit for the second study that will be performed in random order with a 5-14 day interval between each study.
	Data Analysis Plan: The primary endpoint is the difference in EGP during the last hour of EGP measurement in diabetic patients versus diabetic patients without renal transplantation. In our previous study (10) the mean difference in EGP between diabetic subjects who received dapagliflozin versus placebo was 0.70±0.34 (mean ±SD). If renal denervation (i.e., renal transplant diabetic patients) reduces the dapagliflozin-induced rise in EGP, as we hypothesize, by ? 50% compared to dapagliflozin-treated non-renal-transplant diabetic subjects, we computed that 40 subjects (20 per group) are required to demonstrate statistical significance with 90% power and alpha = 0.05. In a secondary analysis we will compare renal transplant diabetic patients treated with dapagliflozin versus placebo. If, as we hypothesize, the renal nerves contribute in part (?50%) to the excessive rise in EGP following dapagliflozin (0.70±0.34), the difference in EGP between the two groups during the last hour of EGP measurement should be reduced by more than 50%. To detect a difference of less than 50% dapagliflozin-treated versus placebo-treated diabetic renal transplant patients with 90% power and alpha=0.05, we computed that 20 subjects are required (10 dapagliflozin and 10 placebo).

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:
Insert procedure here	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 ○ Insert risk here or enter "none"
Insert procedure here	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 ○ Insert risk here or enter "none"
Insert procedure here	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 ○ Insert risk here or enter "none"