COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: SGLT2 Inhibition and Stimulation of Endogenous Glucose Production: Protocol 2

NCT number: NCT02592421

IRB Approval Date: 09/06/2017

Protocol Template Form

Item 1 UTHSCSA Tracking Number	HSC20150668H

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: We will determine the role of pancreatic hormones (increase in plasma glucagon and decrease in plasma insulin concentration) in the stimulation of EGP following SGLT2 inhibition.

Research Design/Plan: After screening, eligible subjects will receive three 5-hour measurements of EGP with administration of study drug after a 3-hour tracer equilibration period. HGP will be measured for 5 hours after drug administration to allow sufficient time for a significant increase in HGP above baseline after dapagliflozin administration (10). In study 1, HGP will be measured for 5 hours after dapagliflozin (10 mg) or placebo administration. Study 2 will be performed under glucose clamp conditions (i.e. maintaining the plasma glucose concentration stable at each subject's fasting level), and Study 3 will be performed under pancreatic clamp conditions (maintaining the plasma glucagon and insulin concentrations constant at the basal level). Subjects will be randomized in a 2:1 ratio; 20 subjects will receive dapagliflozin and 10 subjects will receive placebo. Each study will be performed on a separate day, after a 10-12 hour overnight fast within 1-2 week period.

Methods: Screening visit; Study 1 EGP, Study 2 EGP with Glucose Clamp, Study 3 EGP Pancreatic Clamp

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: EGP is key to understanding normal glucose homeostasis and development of hyperglycemia in T2DM. We and others have demonstrated that glucosuria produced by SGLT2 inhibition stimulates an increase in EGP, suggesting the presence of a strong interaction between the kidney and liver, i.e. "a reno-hepatic axis" that coordinates the regulation of EGP and plasma glucose concentration. The present study will provide evidence for the presence of this previously unrecognized reno-hepatic axis and define the mechanisms which underlie the interaction between the kidney and liver. In addition to providing novel insights concerning the regulation of glucose homeostasis, the present study has direct and important clinical implications. Although SGLT2 inhibitors 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 within the normal range in NGT individuals and prevents hypoglycemia. On the other hand, the increase in EGP in T2DM individuals is "paradoxical" in that it occurs while the plasma glucose concentration is within the hyperglycemic range and quantitatively offsets by ~50% urinary glucose loss (see results below) produced by SGLT2 inhibitors and markedly 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 inhibitors. Our preliminary data suggest an important role for increased glucagon secretion in mediating the "reno-haptic" interaction. In the present study, we will examine whether co-administration of the GLP-1 receptor agonist liraglutide with the SGLT2 inhibitor (dapagliflozin) can prevent the compensatory increase in EGP in response to

	glucosuria. We anticipate that inhibition of glucagon secretion and stimulation of insulin secretion with liraglutide will prevent the compensatory increase in EGP following dapagliflozin administration and produce an additive or even synergistic decrease in the plasma glucose concentration compared to each agent alone. If confirmed by the present study, the clinical implications are enormous and will establish combined SGLT2 inhibitor/GLP-1 receptor agonist therapy as a powerful strategy to reduce the plasma glucose concentration in poorly controlled T2DM individuals.
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: The results of the present study will provide evidence for the existence of such an axis. Moreover, the results of the present study will elucidate the mechanisms that mediate this reno-hepatic interaction. Demonstration of such an axis and definition of the mechanisms that mediate the reno-hepatic interaction will improve our understanding of normal glucose homeostasis and identify novel therapeutic targets for intervention in T2DM individuals to improve their glycemic control.

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**:
Type 2 Diabetic subjects	18-70 yrs old BMI = 25-45 kg/m2 HbA1C < 10% Other than diabetes, subjects must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, T4, EKG and urinanalysis Only subjects whose body weight has been stable (± 3 lbs) over the preceding three months and who do not participate in an excessively heavy exercise program will be included.	Subjects taking drug know to affect glucose metabolism (other than metformin and sulfonylurea) Individuals with evidence of proliferative diabetic retinopathy, plasma creatinine >1.4 females or >1.5 males, 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: Screening Visit. Medical history is obtained and physical exam performed. Blood is drawn for FPG, blood chemistries, lipid profile, HbA1c, and thyroid function. Urine analysis, EKG and pregnancy test are performed.

Study 1: Effect of Dapagliflozin on EGP: EGP will be measured with 3-3H-glucose infusion. The 3-3H-glucose infusion will be started at 6 AM and arterialized blood samples will be drawn from a catheter placed retrogradely in a vein on the dorsum of the hand to measure the basal rate of EGP. The hand will be placed in a box heated to 70°C to ensure arterialization of the venous blood. After a 3 hour tracer equilibration period (at 9 AM) subjects will receive dapagliflozin (10 mg) or placebo, and blood samples will be drawn every 10-20 minutes for 5 hours (until 2 PM) for the measurement of plasma glucose, insulin, C-peptide, glucagon, cortisol, , catecholamine concentrations and [3-3H]-glucose specific activity. Urine will be collected from 6 to 9 AM and from 9 AM to 2 PM. Urinary volume and glucose conc will be measured and the rate of urinary glucose excretion quantitated. The study will end at 2 PM and subjects will be allowed to return home. Basic metabolic panel will be performed during the study to monitor renal function.

Study 2: Effect of Dapagliflozin on EGP With Glucose Clamp: EGP will be measured with 3-3H-glucose infusion as described in Study 1 above. The 3-3H-glucose infusion will be started at 6 AM to measure the basal rate of EGP. After a 3 hour tracer equilibration period (at 9 AM) subjects will receive dapagliflozin (10 mg) or placebo, and the plasma glucose conc will be measured every 5 minutes for 5 hours (from 9AM to 2 PM). A variable infusion of 20% dextrose will be adjusted to clamp the plasma glucose conc at the fasting level (+5%) until the end of the study (2 PM). Blood samples will be drawn every 10-20 minutes for 5 hours (2 PM) for the measurement of plasma glucose, insulin, C-peptide, glucagon, cortisol, catecholamine concentrations and [3-3H]-glucose specific activity. 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 the rate of urinary glucose excretion quantitated. The study will end at 2 PM and subjects will be allowed to return home. Basic metabolic panel will be performed during the study to monitor renal function.

Study 3: Effect of Dapagliflozin on EGP with Pancreatic Clamp: In this study, EGP will be measured as described in Study 1 above and plasma insulin and glucagon concentrations will be clamped at the basal level using the pancreatic clamp technique, while the plasma glucose concentration will be allowed to decrease spontaneously after dapagliflozin and placebo administration. Somatostatin (750 μg/h) infusion will be started at 5:45 AM (15 minutes before the start of [3-3H]-glucose infusion [6 AM]) along with basal infusions of glucagon (0.3 ng/kg.min) and insulin (0.1 mU/kg.min) to replace basal plasma glucagon and insulin concentrations. The somatostatin, glucagon and insulin infusions will be continued for the entire duration of the study (until 2 PM). At 6 AM a prime (0.4 uCi x FPG) – continuous (0.4 uCi/min) infusion of [3-3H]-glucose will be started and continued to 2 PM. At 9AM, subjects will ingest dapagliflozin (10 mg) or placebo (2:1 randomization ratio) and blood samples will be drawn at -30, -20, -10, 5 and 0 minutes before and every 10-20 minutes after drug administration (until 2 PM) for the measurement of plasma glucose, insulin, C-peptide, glucagon, cortisol, catecholamine concentrations, and plasma [3-3H]-glucose specific activity. 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 the rate of urinary glucose excretion will be calculated. The study will end at 2 PM. Basic metabolic panel will be performed during the study to monitor renal function.

Data Analysis Plan: **Data Analysis and Statistical Methods:** Under steady-state postabsorptive conditions, the basal rate of endogenous glucose appearance (Ra = bEGP) equals the 3-^{3}H-glucose infusion rate divided by steady state plasma tritiated glucose specific activity. After drug administration, non-steady conditions for 3-^{3}H-glucose specific activity prevail and the rate of whole body glucose appearance (Ra) is calculated from Steele's equation. During the glucose clamp study, the rate of EGP after drug administration equals Ra minus glucose infusion rate, while, during the pancreatic clamp, Ra = EGP since no cold glucose is infused. The difference in EGP during the last hour of the study (240-300 minute) between subjects receiving dapagliflzin and subjects receiving placebo represents the increase in EGP caused by dapagliflozin (glucosuria-stimulated increase in EGP). The glucosuria-stimulated increase in EGP will be compared among the three studies with ANOVA. Post hoc testing will be performed with a Bonferroni correction for multiple comparisons. The difference in glucosuria-stimulated increase in EGP between study 2 and study 1 represents the contribution

	of the decrease in plasma glucose conc to the increase in EGP, while the difference between study 3 and study 1 represents the contribution of change in islet hormones (insulin/glucagon) to the increase in EGP.

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	**Risks**
example: • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	List the reasonably expected risks under the following categories as appropriate:

EGP measurement with 3-^{3}H-glucose	○ Blood draws and cath placement: o fainting, pain, bruising, swelling, bleeding, anemia or rarely infection where the needle is inserted. o In rare cases the area where the needle is inserted can also become infected or nerves may be damaged, inducing long-lasting abnormal sensations (paresthesia), impaired sensation of touch and persistent pain o Some people may experience mild pain from the catheter. On rare occasions, inflammation of the vein may occur or bruising or black and blueness may occur over the puncture site. Such complications occur in less than 1% of individuals and, if they occur, they usually go away on their own in 2-3 days and are helped by using hot packs. ○ Radiation exposure There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (abnormal cells) or cancer. However, the probability of harm from such risk associated with the amount of radiation exposure received from this study is considered to be low when compared to other everyday risks each member of the general public receives per year, depending on the ○ Heated box: During visits 2, 3, and 4 , the hand is placed in a transparent plastic thermo regulated box heated to 70°C (158°F). Rarely, the hand may suffer a burn or blister. Please tell us if your hand is too hot or feels uncomfortable while in the heated box, in which case your hand will be removed from the box but the test will be continued. ○ Dapagliflozin: o Likely and not serious: o These risks are expected to occur in more than 20 out of 100 subjects: Nausea o o Less likely and not serious: o These risks are expected to occur in 5-20 subjects or less out of 100 subjects: vomiting, diarrhea, constipation, GI upset, dizziness, fatigue, headache, runny nose, and in less than 4 out of 100 individuals low blood sugar (also called Hypoglycemia), urinary tract (about 1%) and genital infections (about 4%) o o Rare and serious: loss of consciousness (or syncopy), decrease in blood pressure (less than 1%), and bladder cancer (less than 0.2%). o o Safety considerations for dapagliflozin and other SGLT2 inhibitors: o Dapagliflozin – is a sodium-glucose cotransporter 2 (SGLT2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus and is a member of a class of antidiabetic medications that work by increasing the glucose excretion in the urine. Dapagliflozin (FARXIGA) has been approved by the U.S. FDA. Side effects reported with dapagliflozin will be summarized below based upon http://www1.astrazeneca-us.com/pi/pi_farxiga.pdf o o Hypotension o Dapagliflozin may cause intravascular volume contraction. Symptomatic hypotension can occur after initiating dapagliflozin particularly in patients with impaired renal function (eGFR less than 60 ±5 mL/min/1.73 m2), elderly patients, or patients on loop diuretics. Subjects with eGFR less than 60 ±5 ml/min/1.73 m2 will not be allowed in the study. Signs and symptoms of hypotension after initiating therapy will be monitored. If a participant experiences any of these, the dapagliflozin will be stopped and the participant will be discontinued from the study. o Renal Function o A small decrease in kidney function (eGFR) and increase in serum creatinine has been observed when dapagliflozin is initially started. The eGFR gradually returns toward normal while the drug is continued and returns to normal if drug is stopped. If the eGFR drops below 60 or if the serum creatinine rises by ≥3.0 mg/dl the dapagliflozin will be stopped and the participant will be discontinued from the study. Sudden kidney injury (acute kidney injury) has happened to people taking dapagliflozin. Talk to your doctor right away if you: o o reduce the amount of food or liquid you drink for example, if you are sick or cannot eat or o o you start to lose liquids from your body for example, from vomiting, diarrhea or being in the sun too long. Seek medical attention immediately if you experience signs and symptoms while taking these medicines such as: o o Decreased urine o o Swelling in your legs or feet o Hypoglycemia o Hypoglycemia (low blood sugar) – can occur with any antidiabetic medication. In studies of dapagliflozin, the frequency of hypoglycemia was less than 5% and similar to that in the placebo group. Subjects will be told to call the investigator if symptoms (sweating, rapid thumping heartbeat, mental confusion) of hypoglycemia occur and to drink a glass of orange juice with a packet of sugar. Any subjects with hypoglycemia will be evaluated for potential causes. If the hypoglycemia recurs, the subject will be discontinued from the study.

	○ Serious urinary tract infections ○ Serious urinary tract infections that may lead to hospitalization have happened in people who are taking dapagliflozin. Tell your doctor if you have any signs or symptoms of a urinary tract infection such as a burning feeling when passing urine, a need to urinate often, the need to urinate right away, pain in the lower part of your stomach (pelvis), or blood in the urine. Sometimes people also may have a fever, back pain, nausea or vomiting. You should seek medical attention and contact your study doctor immediately if you experience any of these symptoms. ○ Genital Mycotic Infections ○ Vulvovaginitis, balanitis and related genital infections were reported in 4.8% and 0.9% of subjects who received dapagliflozin 10 mg and placebo, respectively. Most infections were mild to moderate, and subjects responded to an initial course of standard treatment and rarely resulted in discontinuation from dapagliflozin treatment. These infections were more frequent in females (6.9% and 1.5% for dapagliflozin and placebo, respectively), and subjects with a prior history were more likely to have a recurrent infection. ○ If you experience an genital infection call the study doctor. You will be treated with a local antifungal cream or oral antibiotic therapy. . ○ Volume depletion ○ Dapagliflozin increases the amount of salt and water that is excreted in the urine. Reactions related to volume depletion (including dehydration, hypovolemia, orthostatic hypotension, or hypotension) were reported in 0.8% and 0.4% of subjects who received dapagliflozin 10 mg and placebo, respectively; serious reactions occurred in < 0.2% of subjects balanced between dapagliflozin 10 mg and placebo. ○ Call the investigator if you experience symptoms of volume depletion. ○ Malignancies ○ During clinical trials, the overall proportion of subjects with malignant or unspecified tumors was similar between those treated with dapagliflozin (1.47%) and placebo/comparator (1.35%), and there was no carcinogenicity or mutagenicity signal in animal data. When considering the cases of tumors occurring in the different organ systems, the relative risk associated with dapagliflozin was above 1 for some tumors (bladder, prostate, breast) and below 1 for others (e.g. blood and lymphatic, ovary, renal tract), not resulting in an overall increased tumor risk associated with dapagliflozin. The increased/decreased risk was not statistically significant in any of the organ systems. If you have a prior history of bladder, prostate, or breast cancer please tell us. ○ Bladder Cancer ○ Of 22 clinical studies, newly diagnosed cases of bladder cancer were reported in 0.17% of patients treated with dapagliflozin and 0.03% treated with placebo/comparator. After excluding patients in whom exposure to study drug was less than one year at the time of diagnosis of bladder cancer, there were 4 cases with dapagliflozin and no cases with placebo/comparator. Bladder cancer risk factors and hematuria (a potential indicator of pre-existing tumors) were balanced between treatment arms at baseline. There were too few cases to determine whether the emergence of these events is related to dapagliflozin. . Any subject with a prior history of bladder, prostate, or breast cancer will be excluded from the study. Any subject with hematuria will be excluded from the study. Hematuria will be examined yearly and worked up for etiology. ○ Bone Health ○ Bone mineral density measurements in patients with normal or mildly impaired renal function did not indicate bone loss over a treatment period of one year, and there was no increase in fracture risk in these individuals with normal or mildly impaired renal function. However, it cannot be excluded that with longer periods of treatment there will be a decrease in bone mineral density or increase in bone fracture risk. ○ If you experience a bone fracture contact the study doctor. ○ LDL Cholesterol ○ A small increase in LDL cholesterol of about 4% has been observed in diabetic patients treated with dapagliflozin. ○ Cardiovascular Events ○ No increased risk of cardiovascular events (myocardial infarction, stroke, cardiovascular death) has been observed with dapagliflozin. Any participant who experiences a stroke or myocardial infraction will be discontinued from the study and the IRB and sponsor will be notified. If these individuals should experience a cardiovascular event while taking dapagliflozin, we discontinue them from the study. ○ ○ Hypersensitivity Reactions ○ Severe hypersensitivity reactions, including angioedema and urtricaria have been reported in patient taking dapagliflozin, although a causal association has not been established. Serious anaphylactic reactions and severe cutaneous adverse reactions and angioedema were reported in 0.2% of comparator-treated patients and 0.3% of dapagliflozin-treated patients. If such a reaction occurs in any participants, the dapagliflozin will be stopped and the subject will be discontinued from the study. ○ Pre-clinical Concerns

	○ Dapagliflozin did not induce tumors in either mice or rats at any of the doses evaluated in 2-year carcinogenicity studies. There was no carcinogenicity or mutagenicity signal in animal studies, suggesting that dapagliflozin does not represent a genotoxic risk to humans. ○ ○ The U.S Food and Drug Administration has warned that the use of dapagliflozin (a class of drugs, called sodium-glucose cotransporter-2 (SGLT2) inhibitors) may lead to ketoacidosis, a serious condition where the body produces high levels of blood acids called ketones that may require hospitalization. You should pay close attention for any signs of ketoacidosis and seek medical attention immediately if you experience symptoms such as difficulty breathing, nausea, vomiting, abdominal pain, confusion, and unusual fatigue or sleepiness. Do not stop or change your diabetes medicines without first talking to your prescriber. Your doctor will evaluate for the presence of acidosis, including ketoacidosis, if you are experiencing these signs or symptoms; discontinue SGLT2 inhibitors if acidosis is confirmed; and take appropriate measures to correct the acidosis and monitor sugar levels. ○ If any participant develops any sort of tumors, the dapagliflozin will be discontinued and the IRB and sponsor will be notified. ○ ○ If the study doctor is not available and your symptoms do not improve seek immediate emergency medical care if necessary. ○ amount of radiation exposed to in the past, particularly in the previous year. ○

EGP measurement with 3-3H-glucose with Glucose Clamp	○ Blood draws and cath placement: o fainting, pain, bruising, swelling, bleeding, anemia or rarely infection where the needle is inserted. o In rare cases the area where the needle is inserted can also become infected or nerves may be damaged, inducing long-lasting abnormal sensations (paresthesia), impaired sensation of touch and persistent pain o Some people may experience mild pain from the catheter. On rare occasions, inflammation of the vein may occur or bruising or black and blueness may occur over the puncture site. Such complications occur in less than 1% of individuals and, if they occur, they usually go away on their own in 2-3 days and are helped by using hot packs. ○ Radiation exposure There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (abnormal cells) or cancer. However, the probability of harm from such risk associated with the amount of radiation exposure received from this study is considered to be low when compared to other everyday risks each member of the general public receives per year, depending on the o Heated box: During visits 2, 3, and 4 , the hand is placed in a transparent plastic thermo regulated box heated to 70°C (158°F). Rarely, the hand may suffer a burn or blister. Please tell us if your hand is too hot or feels uncomfortable while in the heated box, in which case your hand will be removed from the box but the test will be continued. ○ Dapagliflozin: o Likely and not serious: o These risks are expected to occur in more than 20 out of 100 subjects: Nausea o o Less likely and not serious: o These risks are expected to occur in 5-20 subjects or less out of 100 subjects: vomiting, diarrhea, constipation, GI upset, dizziness, fatigue, headache, runny nose, and in less than 4 out of 100 individuals low blood sugar (also called Hypoglycemia), urinary tract (about 1%) and genital infections (about 4%) o o Rare and serious: loss of consciousness (or syncopy), decrease in blood pressure (less than 1%), and bladder cancer (less than 0.2%). o o Safety considerations for dapagliflozin and other SGLT2 inhibitors: o Dapagliflozin – is a sodium-glucose cotransporter 2 (SGLT2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus and is a member of a class of antidiabetic medications that work by increasing the glucose excretion in the urine. Dapagliflozin (FARXIGA) has been approved by the U.S. FDA. Side effects reported with dapagliflozin will be summarized below based upon http://www1.astrazeneca-us.com/pi/pi_farxiga.pdf o o Hypotension o Dapagliflozin may cause intravascular volume contraction. Symptomatic hypotension can occur after initiating dapagliflozin particularly in patients with impaired renal function (eGFR less than 60 ±5 mL/min/1.73 m2), elderly patients, or patients on loop diuretics. Subjects with eGFR less than 60 ±5 ml/min/1.73 m2 will not be allowed in the study. Signs and symptoms of hypotension after initiating therapy will be monitored. If a participant experiences any of these, the dapagliflozin will be stopped and the participant will be discontinued from the study. o Renal Function o A small decrease in kidney function (eGFR) and increase in serum creatinine has been observed when dapagliflozin is initially started. The eGFR gradually returns toward normal while the drug is continued and returns to normal if drug is stopped. If the eGFR drops below 60 or if the serum creatinine rises by ≥3.0 mg/dl the dapagliflozin will be stopped and the participant will be discontinued from the study. Sudden kidney injury (acute kidney injury) has happened to people taking dapagliflozin. Talk to your doctor right away if you: o o reduce the amount of food or liquid you drink for example, if you are sick or cannot eat or o o you start to lose liquids from your body for example, from vomiting, diarrhea or being in the sun too long. Seek medical attention immediately if you experience signs and symptoms while taking these medicines such as: o o Decreased urine o o Swelling in your legs or feet o Hypoglycemia o Hypoglycemia (low blood sugar) – can occur with any antidiabetic medication. In studies of dapagliflozin, the frequency of hypoglycemia was less than 5% and similar to that in the placebo group. Subjects will be told to call the investigator if symptoms (sweating, rapid thumping heartbeat, mental confusion) of hypoglycemia occur and to drink a glass of orange juice with a packet of sugar. Any subjects with hypoglycemia will be evaluated for potential causes. If the hypoglycemia recurs, the subject will be discontinued from the study.

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Serious anaphylactic reactions and severe cutaneous adverse reactions and angioedema were reported in 0.2% of comparator-treated patients and 0.3% of dapagliflozin-treated patients. If such a reaction occurs in any participants, the dapagliflozin will be stopped and the subject will be discontinued from the study. ○ Pre-clinical Concerns

	○ Dapagliflozin did not induce tumors in either mice or rats at any of the doses evaluated in 2-year carcinogenicity studies. There was no carcinogenicity or mutagenicity signal in animal studies, suggesting that dapagliflozin does not represent a genotoxic risk to humans. ○ ○ The U.S Food and Drug Administration has warned that the use of dapagliflozin (a class of drugs, called sodium-glucose cotransporter-2 (SGLT2) inhibitors) may lead to ketoacidosis, a serious condition where the body produces high levels of blood acids called ketones that may require hospitalization. You should pay close attention for any signs of ketoacidosis and seek medical attention immediately if you experience symptoms such as difficulty breathing, nausea, vomiting, abdominal pain, confusion, and unusual fatigue or sleepiness. Do not stop or change your diabetes medicines without first talking to your prescriber. Your doctor will evaluate for the presence of acidosis, including ketoacidosis, if you are experiencing these signs or symptoms; discontinue SGLT2 inhibitors if acidosis is confirmed; and take appropriate measures to correct the acidosis and monitor sugar levels. ○ ○ If any participant develops any sort of tumors, the dapagliflozin will be discontinued and the IRB and sponsor will be notified. ○ ○ If the study doctor is not available and your symptoms do not improve seek immediate emergency medical care if necessary. ○ amount of radiation exposed to in the past, particularly in the previous year. ○

EGP measurement with 3-3H-glucose with Pancreatic Clamp	○ Blood draws and cath placement: o fainting, pain, bruising, swelling, bleeding, anemia or rarely infection where the needle is inserted. o In rare cases the area where the needle is inserted can also become infected or nerves may be damaged, inducing long-lasting abnormal sensations (paresthesia), impaired sensation of touch and persistent pain o Some people may experience mild pain from the catheter. On rare occasions, inflammation of the vein may occur or bruising or black and blueness may occur over the puncture site. Such complications occur in less than 1% of individuals and, if they occur, they usually go away on their own in 2-3 days and are helped by using hot packs. ○ Radiation exposure There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (abnormal cells) or cancer. However, the probability of harm from such risk associated with the amount of radiation exposure received from this study is considered to be low when compared to other everyday risks each member of the general public receives per year, depending on the ○ Substances Infusion: Insulin: Insulin is a hormone that naturally is abundant in your body. The only potential side effect known for insulin is hypoglycemia. We will measure your blood sugar every half to 2 hours during the insulin infusion to prevent hypoglycemia from happening. From our previous experience this side effect never happened during our past studies. If you do develop low blood sugar, we can easily make your blood sugar return to normal by giving you glucose. Glucose, glucagon and somatostatin hormones: these substances are abundant in the body and there is no known side effect for their infusion in the amounts we will be giving you. o Heated box: During visits 2, 3, and 4 , the hand is placed in a transparent plastic thermo regulated box heated to 70°C (158°F). Rarely, the hand may suffer a burn or blister. Please tell us if your hand is too hot or feels uncomfortable while in the heated box, in which case your hand will be removed from the box but the test will be continued. ○ Dapagliflozin: o Likely and not serious: o These risks are expected to occur in more than 20 out of 100 subjects: Nausea o o Less likely and not serious: o These risks are expected to occur in 5-20 subjects or less out of 100 subjects: vomiting, diarrhea, constipation, GI upset, dizziness, fatigue, headache, runny nose, and in less than 4 out of 100 individuals low blood sugar (also called Hypoglycemia), urinary tract (about 1%) and genital infections (about 4%) o o Rare and serious: loss of consciousness (or syncopy), decrease in blood pressure (less than 1%), and bladder cancer (less than 0.2%). o o Safety considerations for dapagliflozin and other SGLT2 inhibitors: o Dapagliflozin – is a sodium-glucose cotransporter 2 (SGLT2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus and is a member of a class of antidiabetic medications that work by increasing the glucose excretion in the urine. Dapagliflozin (FARXIGA) has been approved by the U.S. FDA. Side effects reported with dapagliflozin will be summarized below based upon http://www1.astrazeneca-us.com/pi/pi_farxiga.pdf o o Hypotension o Dapagliflozin may cause intravascular volume contraction. Symptomatic hypotension can occur after initiating dapagliflozin particularly in patients with impaired renal function (eGFR less than 60 ±5 mL/min/1.73 m2), elderly patients, or patients on loop diuretics. Subjects with eGFR less than 60 ±5 ml/min/1.73 m2 will not be allowed in the study. Signs and symptoms of hypotension after initiating therapy will be monitored. If a participant experiences any of these, the dapagliflozin will be stopped and the participant will be discontinued from the study. o Renal Function o A small decrease in kidney function (eGFR) and increase in serum creatinine has been observed when dapagliflozin is initially started. The eGFR gradually returns toward normal while the drug is continued and returns to normal if drug is stopped. If the eGFR drops below 60 or if the serum creatinine rises by ≥3.0 mg/dl the dapagliflozin will be stopped and the participant will be discontinued from the study. Sudden kidney injury (acute kidney injury) has happened to people taking dapagliflozin. Talk to your doctor right away if you: o o reduce the amount of food or liquid you drink for example, if you are sick or cannot eat or o o you start to lose liquids from your body for example, from vomiting, diarrhea or being in the sun too long. Seek medical attention immediately if you experience signs and symptoms while taking these medicines such as:

- o Decreased urine
- o Swelling in your legs or feet
- Hypoglycemia
- Hypoglycemia (low blood sugar) – can occur with any antidiabetic medication. In studies of dapagliflozin, the frequency of hypoglycemia was less than 5% and similar to that in the placebo group. Subjects will be told to call the investigator if symptoms (sweating, rapid thumping heartbeat, mental confusion) of hypoglycemia occur and to drink a glass of orange juice with a packet of sugar. Any subjects with hypoglycemia will be evaluated for potential causes. If the hypoglycemia recurs, the subject will be discontinued from the study.
- Serious urinary tract infections
- Serious urinary tract infections that may lead to hospitalization have happened in people who are taking dapagliflozin. Tell your doctor if you have any signs or symptoms of a urinary tract infection such as a burning feeling when passing urine, a need to urinate often, the need to urinate right away, pain in the lower part of your stomach (pelvis), or blood in the urine. Sometimes people also may have a fever, back pain, nausea or vomiting. You should seek medical attention and contact your study doctor immediately if you experience any of these symptoms.
- Genital Mycotic Infections
- Vulvovaginitis, balanitis and related genital infections were reported in 4.8% and 0.9% of subjects who received dapagliflozin 10 mg and placebo, respectively. Most infections were mild to moderate, and subjects responded to an initial course of standard treatment and rarely resulted in discontinuation from dapagliflozin treatment. These infections were more frequent in females (6.9% and 1.5% for dapagliflozin and placebo, respectively), and subjects with a prior history were more likely to have a recurrent infection.
- If you experience an genital infection call the study doctor. You will be treated with a local antifungal cream or oral antibiotic therapy. .
- Volume depletion
- Dapagliflozin increases the amount of salt and water that is excreted in the urine. Reactions related to volume depletion (including dehydration, hypovolemia, orthostatic hypotension, or hypotension) were reported in 0.8% and 0.4% of subjects who received dapagliflozin 10 mg and placebo, respectively; serious reactions occurred in < 0.2% of subjects balanced between dapagliflozin 10 mg and placebo.
- Call the investigator if you experience symptoms of volume depletion.
- Malignancies
- During clinical trials, the overall proportion of subjects with malignant or unspecified tumors was similar between those treated with dapagliflozin (1.47%) and placebo/comparator (1.35%), and there was no carcinogenicity or mutagenicity signal in animal data. When considering the cases of tumors occurring in the different organ systems, the relative risk associated with dapagliflozin was above 1 for some tumors (bladder, prostate, breast) and below 1 for others (e.g. blood and lymphatic, ovary, renal tract), not resulting in an overall increased tumor risk associated with dapagliflozin. The increased/decreased risk was not statistically significant in any of the organ systems. If you have a prior history of bladder, prostate, or breast cancer please tell us.
- Bladder Cancer
- Of 22 clinical studies, newly diagnosed cases of bladder cancer were reported in 0.17% of patients treated with dapagliflozin and 0.03% treated with placebo/comparator. After excluding patients in whom exposure to study drug was less than one year at the time of diagnosis of bladder cancer, there were 4 cases with dapagliflozin and no cases with placebo/comparator. Bladder cancer risk factors and hematuria (a potential indicator of pre-existing tumors) were balanced between treatment arms at baseline. There were too few cases to determine whether the emergence of these events is related to dapagliflozin. . Any subject with a prior history of bladder, prostate, or breast cancer will be excluded from the study. Any subject with hematuria will be excluded from the study. Hematuria will be examined yearly and worked up for etiology.
- Bone Health
- Bone mineral density measurements in patients with normal or mildly impaired renal function did not indicate bone loss over a treatment period of one year, and there was no increase in fracture risk in these individuals with normal or mildly impaired renal function. However, it cannot be excluded that with longer periods of treatment there will be a decrease in bone mineral density or increase in bone fracture risk.
- If you experience a bone fracture contact the study doctor.
- LDL Cholesterol
- A small increase in LDL cholesterol of about 4% has been observed in diabetic patients treated with dapagliflozin.
- Cardiovascular Events
- No increased risk of cardiovascular events (myocardial infarction, stroke, cardiovascular death) has been observed with dapagliflozin. Any participant who experiences a stroke or myocardial infraction will be discontinued from the study and the IRB and sponsor will be notified. If these individuals should experience a cardiovascular event while taking dapagliflozin, we discontinue them from the study.
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	○ Hypersensitivity Reactions ○ Severe hypersensitivity reactions, including angioedema and urtricaria have been reported in patient taking dapagliflozin, although a causal association has not been established. Serious anaphylactic reactions and severe cutaneous adverse reactions and angioedema were reported in 0.2% of comparator-treated patients and 0.3% of dapagliflozin-treated patients. If such a reaction occurs in any participants, the dapagliflozin will be stopped and the subject will be discontinued from the study. ○ Pre-clinical Concerns ○ Dapagliflozin did not induce tumors in either mice or rats at any of the doses evaluated in 2-year carcinogenicity studies. There was no carcinogenicity or mutagenicity signal in animal studies, suggesting that dapagliflozin does not represent a genotoxic risk to humans. ○ ○ The U.S Food and Drug Administration has warned that the use of dapagliflozin (a class of drugs, called sodium-glucose cotransporter-2 (SGLT2) inhibitors) may lead to ketoacidosis, a serious condition where the body produces high levels of blood acids called ketones that may require hospitalization. You should pay close attention for any signs of ketoacidosis and seek medical attention immediately if you experience symptoms such as difficulty breathing, nausea, vomiting, abdominal pain, confusion, and unusual fatigue or sleepiness. Do not stop or change your diabetes medicines without first talking to your prescriber. Your doctor will evaluate for the presence of acidosis, including ketoacidosis, if you are experiencing these signs or symptoms; discontinue SGLT2 inhibitors if acidosis is confirmed; and take appropriate measures to correct the acidosis and monitor sugar levels. ○ ○ If any participant develops any sort of tumors, the dapagliflozin will be discontinued and the IRB and sponsor will be notified. ○ ○ If the study doctor is not available and your symptoms do not improve seek immediate emergency medical care if necessary. ○ amount of radiation exposed to in the past, particularly in the previous year. ○