IRB #	HSC20060247H

COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: PROTOCOL IV. Effect of Plasma Glucose Reduction by Selective SGLT2 Inhibition on Mitochondrial Dysfunction and Impaired Insulin Signaling/Sensitivity in T2DM

NCT number: NCT01439854

IRB Approval Date: 08/10/2016

Unique Protocol ID: 5 R01 DK024092-27/NIH Prot IV

FORM C

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RESEARCH DESCRIPTION

If an item does not apply to your research project, indicate that the question is "not applicable" – do not leave sections blank

For Sections: 1. "Purpose and Objectives"; 3. "Study Design"; and 4. "Study Population," and 5 – 12, you may copy and paste the relevant passages from the sponsor's full protocol or grant application (citing the page number and section is unacceptable). Section 2, "Background" is the only part of this form where you may cite the relevant passages (page number and section) from the sponsor's full protocol or grant application. This section may be used to also describe local standards of practice or add information pertinent to the local IRB review of a multicenter study.

Click once on the highlighted entry in each box to provide your response. Click the item number/letter or word, if hyperlinked, for detailed instructions for that question. If your response requires inserting a table, picture, etc, you may need to first delete the box that surrounds the answer and then insert your table or other special document.

Title of Project:

PROTOCOL IV. Effect of Plasma Glucose Reduction by Selective SGLT2 Inhibition on Mitochondrial Dysfunction and Impaired Insulin Signaling/Sensitivity in T2DM

1. Purpose and objectives. *List the purpose and objectives:*

PRIMARY AIM: To examine the effect of chronic treatment of T2DM subjects with dapagliflozin (a potent, highly specific inhibitor of renal glucose transport [SGLT2]) on mitochondrial gene function/expression and insulin signaling/action. HYPOTHESIS: Chronically elevated plasma glucose levels in T2DM impair mitochondrial function/gene expression and insulin signaling/sensitivity by increasing muscle hexosamine flux and levels of toxic lipid metabolites (FACoA/DAG), secondary to increased malonyl CoA and inhibition of CPT I. Induction of glucosuria with dapagliflozin will normalize/reduce plasma glucose (thereby decreasing muscle hexosamine flux and/or levels of toxic lipid metabolites), leading to improved mitochondrial function and insulin signaling.

SECONDARY AIM: To examine the effect of chronic treatment of T2DM with dapaglifozin on oral glucose tolerance and beta cell function. HYPOTHESIS: Chronically elevated plasma glucose levels in T2DM impair insulin secretion by increasing beta cell hexosamine flux. Correction of hyperglycemia will decrease the beta cell content of hexosamine metabolites, leading to improved insulin secretion.

2. Background.

Describe past experimental and/or clinical findings leading to the formulation of your study.

For research involving investigational drugs, describe the previously conducted animal and human studies.

For research that involves FDA approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.

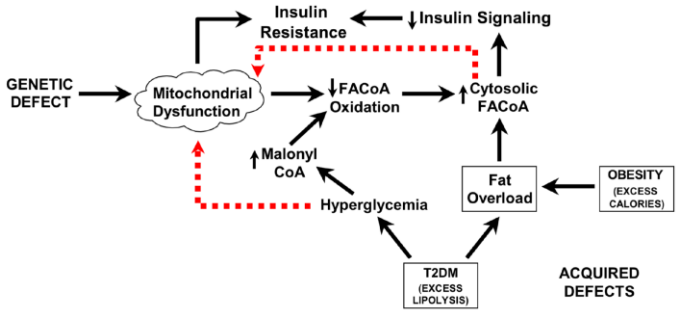
Attach a copy of the approved labeling as a product package insert or from the Physician's Desk Reference.

You may reference sponsor's full protocol or grant application (page number and section) or if none, ensure background includes references.

a. Background

"Glucotoxicity" also has implicated as a cause of insulin resistance and impaired beta cell function in T2DM (1,2). Although abundant support for the glucotoxicity hypothesis has been provided by *in vivo* and *in vitro* (3-14) studies in animals, a rigorous test of this hypothesis in man is lacking. Normalization of day-long plasma glucose levels with intensive insulin therapy has either no effect on or produces only modest improvements in insulin sensitivity/secretion (reviewed in ref #15). However, these studies are difficult to interpret because chronic hyperinsulinemia produces insulin resistance and inhibits endogenous insulin secretion, negating any beneficial effects of reduced plasma glucose. We have shown that treatment of partially pancreatectomized diabetic rats with phlorizin restores normal insulin sensitivity (8) by upregulating the glucose transport system (9). Chronically elevated plasma glucose levels in animals *in vivo* and *in vitro* (15-21) impair mitochondrial function and increase reactive oxygen species (ROS), which would accentuate the defects in fuel (glucose and FFA) metabolism. In mice, glucosamine infusion induces insulin resistance, decreases mitochondrial gene expression, impairs oxidative phosphorylation, and increases ROS (17). Increased ROS activates inflammatory pathways (IkB/NF-kB, p38 MAPK, JNK) and these serine kinases have been shown to inhibit insulin signaling in insulin target tissues (20-28). We propose to test the glucotoxicity hypothesis by chronically reducing the plasma glucose in type 2 diabetic subjects (T2DM) with an inhibitor of renal glucose transport, dapaglifozin, and examining the effect of restoration of normoglycemia on mitochondrial function and insulin signaling/sensitivity. Lastly, we will test the "glucolipotoxicity"

hypothesis (29-31), which states that the toxic effects of elevated plasma FFA on insulin sensitive tissues (i.e., muscle) are magnified in the presence of concurrent hyperglycemia. Thus, high glucose levels increase malonyl CoA, which inhibits CPT I, leading to accumulation of FACoA/DAG, which impair mitochondrial function and inhibit insulin action (Figure 1).



References (from grant application specific to this substudy)

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b. Current practice

Not applicable.

3. Study Design.
Describe the study design (e.g., single/double blind, parallel, crossover, etc.) Consider inserting a scheme to visually present the study design.

Double blind, placebo controlled. However, the study is not testing the drug vs. the placebo with respect to safety or efficacy. The drug is used as a tool to investigate the toxic effect of high blood glucose levels on glucose metabolism.

4. Study Population(s).

You will be drawing subjects from one or more populations. In medical research, for example, a population can be individuals with type 2 diabetes controlled with diet, or a population of healthy individuals. In social behavioral research, a population can be individuals attending an education program, etc.

4.a. How many different populations are you enrolling in this study?	One
4.b. For each different population, provide a short descriptive label: *(e.g., normal-healthy, diabetics, parents, children, etc.)* *Copy and paste additional labels as needed →*	Type 2 diabetes

4.c. For each specific population identified in 4b, provide the following information in the table provided below.
(For studies with more than one population, copy all of table 4.c. and paste to insert additional tables.)

Population # 1	Population Descriptive Label: Type 2 diabetes

(1)	Identify the criteria for inclusion:

(1) Type 2 diabetes mellitus
(2) Fasting plasma glucose = 140-270 mg/dl
(3) BMI = 24-45 kg/m^2
(4) Drug naïve, sulfonylurea treated, metformin-treated, sulfonylurea/metformin-treated
(5) Age = 18-65 years

(6) Stable body weight (± 3-6 pounds) within last 3-6 months

(7) Patients must have the following laboratory values:

Hemoglobin≥ 10.0 g/dl

Serum creatinine ≤2 mg/gl

AST (SGOT) ≤ 3 times upper limit of normal

ALT (SGPT) ≤ 3 times upper limit of normal

(8) Patients must have been on a stable dose of allowed chronic medications for 30 days prior of entering the study.

(9) Patients must be able to communicate meaningfully with the investigator and must be competent to provide written informed consent.

(10) Patients must be of either sex. Females patient must be non-lactating and must be either at least one year post-menopausal or using adequate contraceptive precautions (i.e. oral contraceptives, approved hormonal implant, intrauterine device, diaphragm with spermicide, condom with spermicide) or be surgically sterilize (i.e. bilateral tubal ligation, bilateral oophorectomy). Female patients who have undergone a hysterectomy are eligible for participation in the study. Female patients (except those who have undergone a hysterectomy or a bilateral oophorectomy) are eligible only if they have a negative pregnancy test throughout the study period).

(2)	Identify the criteria for exclusion:

1) Women of child bearing potential who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study
2) Women who are pregnant or breastfeeding.
3) Women with a positive pregnancy test.
4) Urine albumin: creatinine ratio (UACR) > 1,800 mg/g (203.4 mg/mmol Cr)
5) Aspartate aminotransferase (AST) > 3X Upper Limit of Normal (ULN).
6) Alanine aminotransferase (ALT) > 3X ULN.
7) Serum total bilirubin > 1.5 mg/dL
8) Serum calcium value outside of the central laboratory normal reference range.
9) Fasting serum triglycerides > 800 mg/dL (9.04 mmol/L).
10) Hemoglobin ≤ 10.0 g/dL (100g/L) for men; hemoglobin ≤ 9.0 g/dL (90 g/L) for women.
11) Symptoms of poorly controlled diabetes that would preclude participation in this trial including but not limited to marked polyuria and polydipsia with greater than 10% weight loss during the 3 months prior to enrollment, or other signs and symptoms.
12) History of diabetic ketoacidosis or hyperosmolar nonketotic coma.
13) Poorly controlled blood pressure > 160/100 mmHg
14) Any of the following cardiovascular diseases within 6 months of the enrollment visit:

    Myocardial infarction, cardiac surgery or revascularization (coronary artery bypass surgery [CABG]/percutaneous transluminal coronary angioplasty [PTCA]), Unstable angina, unstable congestive heart failure (CHF), CHF New York Heart Association (NYHA) Class III or IV, transient ischemic attack (TIA) or significant cerebrovascular disease, unstable or previously undiagnosed arrhythmia.
15) Congenital renal glucosuria.
16) Donation of blood to a blood bank, blood transfusion, or participation in a clinical study requiring withdrawal of > 400 mL of blood during the 8 weeks prior to the enrollment visit.
17) Malignancy within 5 years of the enrollment visit (with the exception of treated basal cell or treated squamous cell carcinoma of the skin).
18) Known immunocompromised status, including but not limited to, individuals who have undergone organ transplantation or who are positive for the human immunodeficiency virus.
19) Allergies or contraindication to the contents of dapagliflozin tablets.
20) Any unstable endocrine, psychiatric, rheumatic disorders as judged by the Investigator.
21) Subject is, in the judgment of the Investigator, unlikely to comply with the protocol or has any severe concurrent medical or psychological condition that may affect the interpretation of efficacy or safety data.
22) Subject who, in the judgment of the Investigator, may be at risk for dehydration or volume depletion that may affect the interpretation of efficacy or safety data.
23) Subject with any condition which, in the judgment of the Investigator, may render the subject unable to complete the study or which may pose a significant risk to the subject.
24) Subject is currently abusing alcohol or other drugs or has done so within the last 6 months.
25) Subject is a participating investigator, study coordinator, employee of an investigator or immediate family member of any of the aforementioned.

26) Previous participation in a clinical trial with dapagliflozin (BMS-512148) and/or with any other SGLT2 inhibitors.
27) Administration of any other investigational drug within 30 days of planned enrollment to this study.
28) Prisoners or subjects who are involuntarily incarcerated
29) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (e.g., infectious disease) illness.
30.) History of diabetes insipidus.
31.) Severe uncontrolled hypertension defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg.
32.) Replacement or chronic systemic corticosteroid, defined as any dose of systemic corticosteroid taken for > 4 weeks within 3 months of enrollment.
33.) History of bariatric surgery or lap band procedure.
34.) Administration of sibutramine, phentermine, orlisat, rimonabant, benzphetamine, diethylpropion, methamphetamine, and/or phenimedtrazine within 30 days of enrollment.
35.) History of unstable or rapidly progressing renal disease.
36.) Significant hepatic disease, including but not limited to, chronic active hepatitis and/or severe hepatic insufficiency.
37.) Documented history of hepatotoxicity with any medication.
38.) Documented history of severe hepatobiliary disease.
39.) History of hemoglobinopathy, with the exception of sickle cell trait (SA) or thalassemia minor; or chronic or recurrent hemolysis.
40.) Serum Creatinine (SCr) ≥ 2.0 mg/dL unless subject is on metformin then the exclusionary limits will be SCR ≥ 1.50 mg/dL (133 mmol/L) for male subjects; SCr ≥ 1.40 mg/dL (124 mmol/L) for female subjects.
41.) Abnormal free TSH value.
42.) Subjects who have taken thiazolidinediones for within the last three months (90 days)
43.) Subjects who have taken insulin for > 2 weeks within the last year.

(3) **Recruitment Process – identifying potential subjects**

Describe plans about how the population will be **identified** for the purpose of recruiting.

(e.g., database search, personal contacts, referrals, patients under the care of the research team, etc.)

Members of the diabetes care clinic none of whom are members of the research team will identify patients under their care that may be qualified and provide them with contact information for the research team. Subjects are recruited from 4 sources: (i) T2DM who previously have participated in studies; (ii) Diabetes Clinic, Southwest Texas Veterans Health Care System; (iii) Texas Diabetes Institute (Director=RAD), (iv) VA Genetic Epidemiology Study (PI=RAD) (>400 families with strong history of T2DM have been identified).

We also have access to the VA patient database that can search and select patients that are newly diagnosed diabetic patients or have diabetes markers such as HbA1c . We are planning on identifying such potential subjects and then contacting their health care providers for referral. We will send out “dear Doctor” letter informing the providers about our research study and ask them to contact participants that were identified on the VA database. The treating physician will be making the initial patient contact, not the researcher. If the patient is interested, then either the patient will contact the PI or, with the permission of the patient, the PI will be invited to talk with the patient about study participation.

Who will access PHI to **identify** potential participants? *Select one*

☑	Only those with existing legitimate access to PHI will use it to identify potential subjects
☐	There is a need to allow those without existing legitimate access to PHI to use it to identify potential subjects *(submit Form J, HIPAA Waiver)*

(4) **Recruitment Process – first contact**

Describe how **initial contact** will be made with potential subjects

(e.g., researchers will contact potential subjects or subjects will contact the researchers or make appoints to see researchers after learning of the study).

Describe how those making initial contact have a legitimate access to the subjects’ identity and the subjects’ information. *(Consider whether a HIPAA Waiver is needed to disclose PHI to member of the research team who do not have legitimate access.)*

Interested subjects will contact the researchers via telephone. The researchers will return the call and then make an appointment to explain the study to the participant. Researchers will contact potential subjects for those in previous diabetes studies.

If the subjects are identified through the VA database,the treating physician will be making the initial patient contact, not the researcher. If the patient is interested, then either the patient will contact the PI or, with the permission of the patient, the PI will be invited to talk with the patient about study participation.

IRB #	HSC20060247H

(5) **Recruitment process – setting**

Describe the **setting** in which an individual will be initially approached.

(e.g., private room, inpatient unit, waiting area, group setting, over internet, over phone, in public). Also, describe all interaction between the research staff and the potential subject between the time they contact the research team or vice versa and the time they sign a consent form (including pre-screening activities-see instructions for detailed guidance)

Subjects who are interested in participating in studies on the Diabetes Research Unit (DRU) will be asked to come to the 7th floor (Diabetes Research Unit of the Bartter Research Unit) of the South Texas Veterans Health Care System for an interview. During the interview, which will take place in a private room, the study will be explained to subject and the consent form will be reviewed with the subject.

(6) **Recruitment process - advertisements**

Will any advertising be used? ☑ Yes (attach) ☐ No ☐ Pending (will submit an amendment after approval)

If yes, please see Section 4, Form L for instructions on attaching copies of the information to be used in flyers or advertisements. Advertisements must be reviewed and approved by the IRB prior to use.

(7) **Consent Process**

Describe the consent/assent **procedures** that will be used by the research team.

- Include how: information is provided; the consent interview is conducted; the consent is signed.
- Identify the study staff who will conduct the consent interview by their roles (e.g., investigator, research nurse).

* If the consent process of a single subject will involve more than one member of the research team, describe how this process will be coordinated from start to finish.

** If you expect this population will have individuals likely to have diminished decision-making capacity (not including incompetent or impaired decision making capacity), describe the assessment process for determining whether the individual is capable of giving informed consent (i.e., evaluation criteria, time intervals)

Subjects who are interested in participating in studies on the Diabetes Research Unit (DRU) will be asked to come to the 7th floor (Diabetes Research Unit of the Bartter Research Unit) of the South Texas Veterans Health Care System for an interview. During the interview the study will be explained to the subject by the researchers and the consent form will be reviewed with the subject. The subject then will be asked to read the consent form and ask questions about the study. After reading the consent form and all questions and concerns have been answered, the subject will be asked to sign and date the consent form in front of a witness, who will witness the consent procedure. After the consent form is signed by the participant, the witness, and the investigator, a copy of the signed consent will be made and given to the participant. Then inclusion/exclusion criteria will be reviewed with the subject. The subject's height and weight will be measured, and the body mass index (BMI) will be calculated. If there are no exclusion criteria, if the subject meets the inclusion criteria, and if the BMI is within the range described in the protocol, a history and physical exam will be performed. If the history and physical exam are within normal limits, non-fasting screening blood tests (as dictated by the protocol), pregnancy test (for women only), urinalysis and EKG will be obtained.

The consent form will include all of the following:

1. A statement that the study involves research, which includes an explanation of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental;
2. A description of any reasonably foreseeable risks or discomforts to the subject;
3. A description of any benefits to the subject or to others which may reasonably be expected from the research;
4. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject;
5. A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the FDA may inspect the records;
6. For research involving more than minimal risk, an explanation as to whether there is any compensation and an explanation as to whether any medical treatment is available if injury occurs and, if so, what they consist of, or where further information may be obtained;
7. An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and to whom to contact in the event of a research-related injury to the subject;
8. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(8) **Consent Process – time between initial contact and obtaining consent**

Describe the **timing** of obtaining informed consent, whether there is any waiting period between informing the prospective subject and obtaining consent. *(e.g., take consent home, waiting period of X hours, after consulting with family members, etc.)*

IRB #	HSC20060247H

There are no timing issues to obtaining consent in this study. After the study has been explained to the subject, the subject will be asked to read the consent and ask any questions that he/she may have before signing the consent.

(9) Describe measures taken to **minimize** the possibility of coercion or undue influence during consent.

After explaining the study, subjects will be allowed to read the consent freely and with sufficient time. Subjects will not be pressured in any way to enter the study. We will explain that the study is voluntary and that, if they decide not to participate (or exit the study), they (and their family members) will not be punished in any way, and this will not affect their eligibility to enter future studies or to seek medical treatment at the Audie L. Murphy Hospital.

(10) Will subjects from this population be assigned to different research groups? *(e.g., treatment and control group)*

☑ Yes ☐ No

If yes, list the groups by inserting a short descriptive title for each group.
E.g., experimental group A, B, etc., control group, etc. - these labels are needed for the Risk: Benefit Analysis section
Dapagliflozin – treated diabetic subjects
Placebo-treated diabetic subjects

5. Informed Consent for Research Involving Non-English Speaking Subjects – *choose either A, B or C*

A. ☐ **N/A**. The primary investigator for this study will request a waiver of consent for all subjects in this study. (go to #6)

☐ **N/A** This study does not involve interaction with living individuals; *(limited to use of identifiable information)*. (go to #6)

OR

B. ☑ **Only individuals who speak English will be enrolled**. *(if checked select one of the two statements below)*

☑ There is **no** expected direct benefit for those participating. (go to #6) Although it is expected that dapagliflozin will reduce the blood glucose concentration, the treatment period is short (2 weeks) and no clinical term benefit is expected. There is no expected benefit in the group receiving placebo for 2 weeks.

☐ There is an expected direct benefit for those participating. Excluding non-English speaking individuals is acceptable because: *(insert the rationale for excluding this population below* -- then go to #6)

OR

C. ☐ **Individuals who do not speak English will be enrolled**.
The translated consent will be submitted to the IRB:
Select one Form B, item 12 should be checked

☐ Immediately following approval of the English consent.
(go to c(1) and c(2) below)

☐ Only after a potential non-English speaking participant is identified. Since this plan will delay enrollment pending IRB approval of a translated consent, provide justification that prospective non-English speaking subjects will not be excluded from beneficial research.
Choose one of the choices below:

☐ There is **no** expected direct benefit for those participating. (go to c(1) and c(2) below)

☐ There is an expected direct benefit for those participating.
Provide justification why the delay is acceptable below, then (go to c(1) and c(2)below)

Insert the reason a delay is acceptable here

C (1) If you are recruiting non-English speaking subjects, Describe the process for obtaining informed consent from prospective subjects in their respective language (or the legally authorized representative's respective language).

Describe here

C (2) In order to ensure that individuals are appropriately informed about the study when English is their second-language, describe a plan for evaluating the level of English comprehension, and the threshold for providing a translation, or explain why an evaluation would not be necessary.

Describe here

IRB #	HSC20060247H

6. Research Plan / Description of the Research Methods:

6.a. Provide a **comprehensive narrative** describing the **research methods**.

Provide the **order in which tests/procedures will be performed**, the **setting** for these events and a description of the **methods used to protect privacy** during the study.

Provide the **plan for data analysis** (include as applicable the **sample size calculation)**

The study participants will receive **5 visits**, during which we will perform baseline studies to assess insulin sensitivity in skeletal muscle and liver of the study participants before and after 2 weeks of dapagliflozin treatment. For this purpose, subjects will be screened for eligibility to participate in the study at **visit 1** and also be given the option of completing the OGTT and DEXA during visit 1 after consented and screened. If eligible subjects do not complete the OGTT and DEXA during visit 1 they will perform 75-gram OGTT and DEXA scan in **visit 2**. In **visit 3**, subjects will receive a baseline insulin clamp (insulin sensitivity) test with muscle biopsy. During this visit also we will start the dapagliflozin treatment. Between days 14-19, subjects will return to the BRU for **visit 4** for a repeat OGTT. Subjects will return to the BRU for **visit 5**, between days 16-21 in which the insulin clamp will be repeated. The procedures to be performed during each visit are detailed in the table below.

TIME AND EVENTS SCHEDULE

	Visit 1	Visit 2	Visit 3					(Day 14*) (D 14-19)	(Day 16*) (D 16-21)
Eligibility Assessments									
Informed Consent	X								
Inclusion/Exclusion	X								
Medical History	X							X	
Safety Assessments									
Physical Exam	X							X	
Vital Signs	X							X	
Adverse Events Assessments								X	X
Lab Tests	X								X
Pregnancy Test	X								X
Efficacy Assessments									
OGTT	X*	X						X	
DEXA	X*	X							
Insulin Clamp			X						X
Muscle Biopsies (1-2 per Visit)			X						X
HGP (3-3H-glucose turnover)									
24-h urine									X
Blood tests *BMP		x	x					X	X
24h CGMS								X	
Start DAPA/Placebo			x						
Randomize			x						
Dispense Drug			x						

* Option of completing OGTT and DEXA during visits 1 or 2 after Eligibility Assessment

Experimental Design: Prior to randomization subjects will receive: (i) OGTT; (ii) DEXA; (iii) insulin clamp with 3-^{3}H-glucose, indirect calorimetry, vastus lateralis muscle biopsies at -60 minutes and 240 minutes after start of the insulin. The OGTT and insulin clamp will be performed in the morning following an overnight fast. The OGTT, DEXA, and insulin clamp will be performed over a 3 day to 2 month interval; (iv) plasma fructosamine, HbA_{1C}, lipids, hsCRP, electrolytes; (v) 24-h urine collection fo glucose, Na^+, K^+, Cl^- and creatinine excretion x -1;

T2DM subjects will be randomized (2:1) in double blind fashion to receive dapagliflozin, 10 mg/day for 2 weeks (n=20) or placebo (n=10). The first dose of dapagliflozin will be given during visit 3. All studies (except the DEXA and the number of muscle biopsies during the second euglycemic insulin clamp; 1-2) will be repeated after 2 weeks. The 24-h urine collection for glucose, Na^+, K^+, Cl^-

and creatinine excretion will be repeated on Visit 5 (final visit, the day of the insulin clamp).

A number of techniques will be employed in this study and they are described in the detail below.

I. OGTT: After obtaining 4 baseline samples, subjects ingest 75 g of glucose. Blood is obtained q 15 min for 2 h for plasma glucose, insulin, C-peptide and FFA conc (34).

II. Euglycemic Insulin Clamp (insulin sensitivity test)**.** All subjects will receive a 240 min euglycemic insulin (80 mU/m^2·min) clamp (36) to increase plasma insulin by ~100 uU/ml. During the 180 min prior to the insulin clamp, a primed (20 uCi x FPG/90)-continuous (0.20 uCi/min) infusion of 3-^{3}H-glucose is started (37,38). During the insulin clamp, no glucose is infused until the plasma glucose declines to 100 mg/dl, at which level it is maintained. During the baseline/insulin clamp periods continuous indirect calorimetry is performed (39,40) to quantitate rates of glucose/lipid oxidation, and blood samples are obtained q 5-15 min for plasma insulin, C-peptide, glucose, FFA, glycerol concentrations and plasma 3-^{3}H-glucose sp act (38,39). Data Analysis. Under steady state postabsorptive conditions, the rate of endogenous glucose appearance (Ra) = 3-^{3}H-glucose inf rate (DPM/min) ÷ steady state plasma glucose sp act (DPM/mg). During the insulin clamp, non-steady conditions prevail and Ra is calculated from Steele's equation. Endogenous (primarily hepatic) glucose production = Ra minus exogenous glucose inf rate. Total glucose disposal (TGD) = EGP + exogenous glucose inf rate. Glucose and lipid oxidation are determined from NPRQ using standard equations (38,39). TGD - glucose oxid = non-oxidative glucose disposal, which agrees closely with muscle glycogen synthesis determined by ^{13}C NMR (41).

III. Vastus Lateralis Muscle Biopsy. Percutaneous biopsy of the vastus lateralis muscle (~200-250 mg) is performed 60 min before and/or 240 min after start of insulin (42-44). Muscle biopsy samples are immediately frozen in liquid nitrogen and stored at –80˚C for analysis. **The baseline (-60 min)** biopsy is used to examine insulin signaling events (IR and IRS-1 PY/protein content; assoc of p85/PI-3 K activity with IRS-1, total PI-3 K activity) as previously described (43-45). In time course studies, we have shown that IRS-1 PY, PI-3 K activity and p85/PI-3 K activity assoc with IRS-1 are maximally stimulated at 30 min. The following measurements will be performed on the **baseline (-60 min) and 240 min** biopsies: (i) PDH activity (glycolytic enzyme), citrate synthase (TCA cycle), beta-HAD (beta oxidation enzyme), cytochrome c oxidase (oxidative enzyme), glycogen synthase (glycogenic enzyme). Details of these assays have previously been published (43,44,46-49); (ii) UDP-N-acetylglucosamine (UDP-GlcNAc) and glutamine: fructose-6-amido transferase using previously published methods (50,51); (iii) gene expression using DNA microarrays (Affymetrix) (42); (iv) malonyl CoA content measured with a radioactive method using fatty acid synthase (30); (v) IKK/IkB/NF-kB signaling by measuring NF-kB DNA binding activity (52,53) and IkBα protein by Western blotting. Changes in NF-kB binding correlate inversely with IkBα protein content (52,54); (vi) p38 MAPK and JNK will be quantitated by Western blot analysis (24,26); (vii) *ex vivo* measurement of mitochondrial function using confocal laser microscopy (see below). The following measurements will be made only on the **-60 min muscle biopsy**: (i) FACoA and DAG content (see below); (ii) mitochondrial DNA content using Q-RT-PCR (55).

IV. Microarray Analysis. Muscle biopsy specimens are homogenized directly in RNAStat solution and RNA pellets are stored in ethanol/sodium chloride solution at –80ºC. Prior to use, total RNA is purified with RNeasy and DNase I treatment (Qiagen). For microarray analysis (42), RNA is prepared for hybridization to Affymetrix HG-U133A arrays according to the manufacturer's instructions (see detailed description in ref 42 and 56).

V. Quantitative TaqMan RT-PCR. Muscle expression of genes of interest (PGC-1, NRF-1, genes coding for mitochondrial energy metabolism [glycolytic/TCA cycle genes, mitochondrial respiratory chain, etc]) that are found to be down/upregulated with microarray analysis will be quantitated using one-step Q-RT-PCR from the same total RNA used for microarray analysis (42,56). All gene expression/Q-RT-PCR measurements will be performed by Dr. Christopher Jenkinson (Assoc Prof, Diabetes Div; Director, Core Molecular Genetics Laboratory [CMGL]) who has performed all mRNA expression/PCR measurements described in previous publications from our lab. The CMGL is housed within Diabetes Division.

VI. Intramyocellular Lipids: FACoAs and Diacylglycerol. Muscle LC-FACoA levels will be quantitated by Prof Gerald Shulman (Yale Med Sch, New Haven, CT) using liquid chromatography tandem mass spectrometry (57,58). Muscle diacylglycerol levels are determined as described by Preiss (59). Following lipid extraction with chloroform: methanol: PBS+0.2% SDS (1:2:0.8), diacylglycerol kinase and [γ-^{32}P] ATP (15 uCi/umol cold ATP) are added and reaction is stopped with chloroform: methanol (2:1). Samples are run on thin-layer chromotgraphy plates in chloroform:acetone: methanol:acetic acid:water (100:40:20:20:10) and the DAG band is counted.

VII. *Ex Vivo* Measurement of Mitochondrial Function. Mitochondrial function will be examined using confocal optical microscopy (63,64). During mitochondrial respiration, energy is liberated to drive ATP synthesis at 3 coupling sites where protons are pumped out across the mitochondrial inner membrane, generating an electrochemical gradient of protons ($\Delta\mu_{H^+}$) or equivalently, a protonmotive force ($\Delta p = \Delta\mu_{H^+}/F$) (65), which drives ATP synthesis. Δp is comprised of two components: membrane potential ($\Delta\psi$) and pH gradient (ΔpH) and $\Delta p = \Delta\psi - 60\Delta$pH (65,66). Using laser confocal microscopy, both $\Delta\psi$ and ΔpH of mitochondria in tissue culture, myocytes and muscle tissue (63-67) can be measured. For observation of $\Delta\psi_m$, muscle tissue (-60 and 240 min biopsies) is placed in DMEM culture medium with tetramethylrhodamine ester (TMRE; 500 nM, 30 min, 37ºC), which accumulates into polarized mitochondria in response to $\Delta\psi_m$ (68) (see preliminary data). Measurement of ΔpH also is of great interest. Cytosolic pH (pH_i) is monitored using BCECF ratio imaging, as previously described (69,70), while mitochondrial pH (pH_m) is monitored using Carboy-SNARF-1-AM (20 M, 2 h, 4ºC). Tissue is loaded with either BCECF or Carboxy-SNARF-1-AM and incubated for 4-6 h at 37ºC. Under these conditions, cytosolic dye is pumped out of cells, leaving just labeled mitochondria.

VIII. Beta Cell Function. Glucose and insulin areas under OGTT curve (AUC) are calculated by trapezoidal rule. Insulin secretion rate (ISR) is obtained by deconvolution of plasma C-peptide curve (71). Insulin resistance (IR) is determined with the insulin clamp. The insulin secretion/insulin resistance index or "disposition index" ([ΔISR ÷ ΔG]/IR) (35) provides a physiologically relevant measure of beta cell function (72).

IRB #	HSC20060247H

IX. Plasma Adipocytokine Conc. Before and after dapagliflozin, we will measure plasma levels of adiponectin, resistin, IL-6, IL-1, TNFα, leptin, and PAI-1 (73-75).

Limitations, Interpretive Problems, Expected Results: Based upon studies performed in diabetic rats, dogs, and humans, we expect dapaglifozin to inhibit renal tubular glucose transport, induce glucosuria, and cause sustained reduction in plasma glucose. As plasma glucose decreases to near normal values, glucosuria will diminish but we anticipate that improved glycemic control will persist because: (i) basal HGP is reduced (in diabetic animals, chronic hyperglycemia upregulates hepatic G-6-phosphatase and restoration of normoglycemia decreases G-6-phosphatase) (76); (ii) post-prandial glucosuria limits the rise in plasma glucose. Reduced mean day-long glucose will be documented by continuous 24 h blood glucose monitoring (CGMS). We do not expect significant changes in body weight/composition (DEXA) during the short two week study duration. Weight will be monitored and, if necessary, caloric intake adjusted to maintain weight constant. If the glucotoxicity hypothesis is correct, dapaglifozin should enhance peripheral (muscle) and hepatic insulin sensitivity. From muscle biopsy, we will define whether improved insulin sensitivity results from enhanced glucose transport/phosphorylation or increased insulin signaling. If "glucotoxicity" exerts its deleterious effects through "lipotoxicity" (i.e., increased malonyl CoA, inhibition of CPT I, increased muscle FACoA), we expect to observe decreased muscle FACoA/malonyl CoA levels. If "glucotoxicity" is mediated, in part, via increased hexosamine flux, we expect decreased muscle UDG-GlcNAc and GFAT activity. Based upon animal studies (8,17), we hypothesize that diminished hexosamine flux (or some other, as of yet, undefined mechanism) will lead to improved mitochondrial function (^{31}P-NMR, enzyme activities, TMRE)/gene expression (microarray analysis, PCR). To the extent that glucotoxicity contributes to beta cell dysfunction (1-6), we hypothesize that reduced day-long glycemia will enhance insulin secretion (ΔISR/ΔG÷IR index).

Data Analysis/Sample Size Calculation.

Data will be analyzed using a two-way ANOVA for repeated measurements. Before and after is a repeated-measurements factor and treatment (dapagliflozin) is a between group factor. The interaction, that is, how the differences between before and after vary by treatment is of interest. Comparisons of these differences among treatments following the ANOVA will use a multiple comparison test.

Power for 25% differences before vs. after treatment with dapagliflozin for T2DM patients in a paired two-tailed test with α=0.05 and 15 subjects/group (column A).

Variable	Mean Diabetics	Difference (%)	Power A
Glucose disposal	2.40±0.74	25	0.985
Glu Oxidation	1.20±0.45	25	0.924
Gly Synthesis	1.20±0.35	25	0.992
ΔI/ΔG ÷ IR	1.90±0.26	25	0.999
ΔIRS tyr phos	0.08±0.028	25	0.953
PI3K assoc IRS-1	0.03±0.009	25	0.988
P85 assoc IRS-1	0.01±0.003	25	0.988
Total PI3K	0.15±0.058	25	0.908
Gly Synthase FV	0.08±0.025	25	0.982
Citrate Synthase	42.00±9.67	25	0.999
IkB protein	50±15	25	0.985
Muscle FACoA	3.90±0.69	25	0.999
Mito DNA	470±63	25	0.999
TMRE	2245±304	25	0.999
Muscle Fat (MRS)	2.20±0.74	25	0.966
PGC-1α	16.00±4.20	25	0.998
PGC-1β	2.60±0.46	25	0.999
NRF-1	4.00±0.54	25	0.999
Muscle ATP (MRS)	5.30±0.92	25	0.999

All procedures will be performed at the BRU and clinical privacy practices will be followed.

IRB #	HSC20060247H

6.b. List of the study intervention(s) being tested or evaluated under this protocol

☐ **N/A -** this study does not test or evaluate an intervention. Skip to item 6.d.

#	Study intervention(s) being tested or evaluated under the protocol *Add or delete rows as needed*	Local Standard Practice Indicate whether the intervention is considered acceptable practice locally
1	**Dapagliflozin**	☐

6.c. Risk:Benefit Analysis of study interventions being tested or evaluated under this protocol

For each study intervention identified in section 6b above, complete a risk:benefit analysis table.

(Two tables are provided, copy & paste additional tables as needed or delete both tables if this study does not test an intervention)

IRB #	HSC20060247H

6.c. **Study Intervention #1** Dapagliflozin (21 days)	
List each group exposed to this intervention on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this intervention. (Benefits can be directly from the intervention or from a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
All Subjects	No intended benefit

For this intervention, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)
Do not delete frequency. The need to know what to report promptly later = a requirement to estimate frequency (Instructions).

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 out of 100 subjects.	• None	• None
	Not serious	**Serious**
Less likely These risks are expected to occur in 5-20 subjects or less out of 100 subjects.	Due to increased sugar in the urine: • 8% of women and 2.8% of men have experienced genital fungal infections. These infections have been mild and usually disappear spontaneously or with antibiotics. • 7% of the people have experienced a bacterial urinary tract infection.	• none
		Serious
Rare These risks are expected to occur in less than 5 subjects out of 100		• *Increased AST, ALT, TB*: If AST/ALT are increased 3 x ULN or total bilirubin is increased 1.5 x ULN after day 16, subjects will not be allowed into Protocol VI and they will be followed until the abnormal lab value resolves. • Hyponatremia due to increased urine: If plasma sodium is < 125 meq/L after day 16, subjects will not be allowed into Protocol VI and they will be followed until the abnormal lab value resolves. • Elevated Creatinine Kinase. If plasma CK is > 10 x ULN after day 16, subjects will not be allowed into Protocol VI and they will be followed until the abnormal lab value resolves. • Elevated Creatinine: If plasma creatinine is > 2.0 mg/dl after day 16, subjects will not be allowed into Protocol VI and they will be followed until the abnormal lab value resolves • *Hypoglycemic Symptoms:* If subjects experience symptoms of hypoglycemia or a blood glucose < 54 mg/dl and they are on a sulfonylurea, the sulfonylurea will be stopped and the study continued. If hypoglycemia continues to occur or if the blood glucose is < 54 mg/dl, the subject's participation in the study will end. If the subject experiences a major hypoglycemic episode (hypoglycemic symptoms with blood glucose less than 54 mg/dl and associated with impaired mental status or requiring third party assistance to recover, the subject's participation will end and the subject will be followed until the hypoglycemia resolves. • *light-headedness secondary to low blood pressure*

		• Sudden kidney injury (acute kidney injury) has happened to people taking dapagliflozin. Talk to your doctor right away if you: o reduce the amount of food or liquid you drink for example, if you are sick or cannot eat or 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 •
		•

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.

IRB #	HSC20060247H

6.d. List of ALL other research procedures or components not listed in table 6.b.
All of the research procedures for this study should be listed in either table 6.b. or 6.d.

Consider grouping similar procedures under a single component. E.g., blood work, CT = safety assessments)
(Click here for example)

#	Research component • **individual procedures** *example:* **Eligibility Assessments** • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	Column A **Local Standard Practice** Indicate the number of times each procedure will be performed as stipulated in the research plan **that would be done as part of standard practice.**	Column B **Research Only** Indicate the number of times each procedure will be performed solely for research purposes *(any performed outside frequency or timing for acceptable local practice)*	Column C Place a check if the procedure will be performed at the VA	Column D **Risks** List the reasonably expected risks under the following categories as appropriate: • Serious and likely; • Serious and less likely; • Serious and rare; • Not serious and likely; • Not serious and less likely
1	**Eligibility Assessments**				
	Consent	0	1	X	none
	History	0	2	X	none
	PE	0	2	X	none
	Inc/Exc Criteria	0	1	X	none
	EKG	0	2	X	none
2	**Laboratory Test (total 24 ml)**				
	HbA1C, fructosamine, Chem 20, CBC, plasma lipids, hsCRP, adipocytokines,	0	2	X	Not serious and likely: Mild pain and bruising at puncture site
	TSH/T4, PTT, PT, INR	0	1	X	none
	CK and Uric Acid	0	1	X	none
	Serum Pregnancy	0	2	X	same
	Urine Analysis	0	2	X	none
	Plasma electrolytes	0	3	X	none
	BMP		2		Not serious and likely: Mild pain and bruising at puncture site
	24 hour urine: glucose, Na^+, K^+, Cl^-, and creatinine	0	1	X	
3	**OGTT**				
	Glucose drink	0	2	X	Not serious and likely: Mild nausea
	Venipuncture - BS	0	2	x	Not serious and likely: Mild pain and bruising at puncture site Blood loss about 94 ml
4	**Insulin clamp**	0	2	x	Not serious and rare: Hypoglycemia is possible.
	IV catheter placement & blood draw	0	2	x	Not serious and likely: Mild pain and bruising at puncture site Blood loss: 190 ml of blood are drawn during the insulin clamp.
	Tritiated glucose	0	2	x	Not serious and likely: Radiation: The radiation exposure is small (8.41 mrem total radiation exposure per clamp), which is well within safety guidelines.
	Heated box	0	2	x	Serious and rare: Skin burn from the hot box: We have had 1 individual with mild skin burn while the hand was in the hot box.
	Indirect Calorimetry	0	2	x	Serious and rare: Claustrophobia .

	Heated box	0	2	x	Serious and rare: Skin burn from the hot box: We have had 1 individual with mild skin burn while the hand was in the hot box.
5	**Muscle Biopsy (X4)**				
	Thigh muscle bx	0	3-4	x	Not serious and likely: Pain: At the time of biopsy, subjects may feel mild pain, discomfort, or pressure (variably described by different subjects) for about 5-10 seconds. Pain or discomfort ceases as soon as the biopsy needle is withdrawn. **Not serious and rare:** Local hematomas: Local hematomas occur rarely and they resolve spontaneously within 2 weeks. Numbness: Rarely non-clinically evident numbness or altered sensation can occur at the biopsy site, which is transient (about 1 month). Infection: There is a very low risk of infection in the biopsy area. We have not had any infection in our protocols. Allergy: Allergic reactions to the local anesthetic (lidocaine) are extremely rare, but could include dermatitis, swelling, or hives. Serious and rare: Permanent nerve damage: Permanent nerve damage manifested as localized numbness or decreased sensation is possible, but very rare.
6.					
7.					
8	**DEXA for body composition**				
	radiation	0	1	X	Not serious and likely: Subjects will be exposed to a small amount of radiation

IRB #	HSC20060247H

7. Safety Precautions. *(Describe safeguards to address the serious risks listed above.)*

a. Describe the procedures for protecting against or minimizing any potential risks for each of the more than minimal risk research procedures listed above.

Risks will be minimized by a careful screening process including medical history, physical exam, and review of complete blood count, coagulation tests, chemistry and electrocardiogram. The presence of experienced personnel, and of at least one physician, at all times is also critical to reducing risks.

Insulin Clamp: Plasma glucose concentration will be determined at 5 minute intervals throughout the period of insulin/glucose administration. The 20% dextrose infusion will be adjusted in order to maintain euglycemia (100 mg/dl) and to avoid hypoglycemia.

Muscle biopsies: To avoid local hematoma formation, pressure is applied for 30 min after the biopsy. To avoid infection, the procedure is performed under sterile conditions. Our group has experience with over 1000 biopsies.

Blood loss: the total amount of blood drawn will be about 522 ml per subject. The subjects will be told that they should not donate blood for two months after the study. Any subjects with a hematocrit of less than 35% will not be studied.

Radiation Exposure: Radiation exposure is within acceptable guidelines established by the Radiation Safety Committee. Subjects will be questioned about prior radiation exposure and they will be told that they should not participate in any study involving radiation exposure for at least one year.

Potential Side Effects of Dapagliflozin: Subjects who experience a hypoglycemic episode will not be allowed to continue in the study. Subjects who experience an elevated plasma creatine, creatine kinase, AST, ALT, total bilirubin or reduced plasma sodium (see section 6d) they will be followed until the abnormal lab value resolves.

b. Where appropriate, discuss provisions for ensuring necessary medical or professional intervention in the event of adverse events, or unanticipated problems involving subjects.

Any expected or unexpected adverse events will be assessed by Dr. DeFronzo or a co-investigator. Any necessary immediate medical care will be arranged in conjunction with the subject's wishes.

c. Will the safeguards be different between/among groups?

☐ Yes ☑ No

If yes, describe here

8. Confidentiality of the Research Information

a. Specify **where** the data and/or specimens will be stored and **how** the researcher will **protect the confidentiality** of subject information.

Paper data will be stored in STHCS BRU Room 728 in a locked cabinet in a locked room per VHA regulations. Electronic data will be stored the VA Server (folder name Folder name Dapa-Mito) per VHA regulations. De-identified Specimens will be stored in the Diabetes Division until processing (Room 3,380S) in a locked room per UTHSCSA regulations.

The Specimens will be stored in a restricted access area in Dr. DeFronzo's laboratory in the Diabetes Division.

b. Will all electronic data be stored in accordance with the institution's information security policy and encryption standards?

☑ Yes ☐ No, if no explain below

IRB #	HSC20060247H

9. Payment. (payment of subjects should be included in the consent form)

a. Describe the incentives (e.g., inducements) being offered to subjects for their time during participation in the research study.

There are no inducements.

b. If monetary compensation is offered, indicate how much the subjects will be paid and describe the terms and schedule of payment.

IRB policy requires a provision for providing partial payment to subjects who withdraw before the completion of the research. For *VA research, payment to human subjects participating in research is prohibited when the research is integrated with the patient's medical care and when the research makes no special demands on the patient beyond those of standard medical care. Payment may be permitted, with IRB approval, under certain circumstances. Consult with the VA R&D Office to discuss payment of subjects.*

As reimbursement for time and travel expenses, subjects will receive $40 for the study qualification visit, $40 for each oral glucose tolerance test and $250 for each insulin sensitivity (clamp) test. Subjects will receive $20 for the DEXA test. If subjects should decide to stop their participation in the middle of the oral glucose tolerance test or the insulin sensitivity test, or the radioactive glucose studies while on the BRU, they will receive $15 per hour for that part of the test that they have completed. Subjects also will receive $90 per day for each of the 3 days that they are on the BRU. Subjects will not have to pay anything for tests that are done as part of their participation in the study. If subjects complete the entire study, they will receive $1090.

10. Costs to Subjects. (costs to the subject should be included in the consent form)

a. Describe any costs for care associated with research (including a breakdown of standard of care procedures versus research procedures), costs of test drugs or devices, and research procedure costs that are the subject's responsibility as a consequence of participating in the research.

No cost to subjects.

b. Describe any offer for reimbursement of costs by the sponsor for research related injury care. (Attach a copy of the section of the clinical trial agreement or contract describing research related injury care – the information in this section must match the injury section of the consent form).

If subjects are a Veteran and in the event that they sustain an injury or illness as a result of their participation in this VA approved research study, all medical treatment (emergency as well as medical treatment beyond emergency care) will be provided by the VA. Subjects will be treated for the injury at no cost to the subject. However, no additional compensation has been set aside. Subjects have not waived any legal rights or released the hospital or its agents from liability for negligence by signing this form.

In the event of a research related injury or if subjects experience an adverse reaction, they should immediately contact the study doctor at telephone number listed on page 13 during the day and after business hours. If subjects need emergency hospitalization in a private hospital because they are unable to come to the VA, they should have a family member or friend contact the study doctor so that the VA can coordinate care with the private hospital.

11. PI-Sponsored FDA-Regulated Research

If the PI is the IND/IDE holder, or has agreed to perform any of the IND/IDE holder's sponsor obligations, the PI is considered a sponsor (sponsor investigator) and must meet additional requirements. (Form O, O-1 and P provide details)

[see Office of Clinical Research policies]

☑ N/A. The PI is not the IND or IDE holder, and has not agreed to perform sponsor obligations

a. Has the PI completed the CITI module: Conducting Investigator-Initiated Studies According to FDA Regulations and Good Clinical Practices?

☑ Yes ☐ No. If no, complete the training prior to submitting this protocol

b. Describe the PI's experience/knowledge/training related to serving as a sponsor-investigator.

Over the last 35 years, Dr. DeFronzo has performed multiple sponsor-investigator initiated studies and has had 35 years of consecutive NIH funding for clinically related research. During these studies there have been no serious adverse events. Dr. DeFronzo has completed the CITI module training (09/28/2010). Dr. DeFronzo has had 11 IND's approved by the FDA (both active and some now inactive) and the FDA never has denied renewal of any of these INDs. There have been no serious adverse events associated with any of these INDs.

FORM C

IRB #	HSC20060247H

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. This summary will also be needed in future IRB Progress Reports.
DO NOT EXCEED THE SPACE PROVIDED.

Purpose/Objectives: To examine the effect of a chronic decrease in plasma glucose in T2DM subjects using dapagliflozin on insulin sensitivity/signaling, mitochondrial function, oral glucose tolerance, and beta cell function.

Research Design/Plan : Screen approximately 75 subjects in order to obtain 30 completers that will receive an OGTT and euglycemic insulin clamp with vastus lateralis muscle biopsies and then be randomized to dapagliflozin (n=20) or placebo (n=10). After 2-3 weeks the OGTT and euglycemic insulin clamp will be repeated. [A large attrition rate is expected due to the complexity of the protocol and the inclusion/exclusion criteria.]

Methods: (1) 2-hour oral glucose (75 grams) tolerance test with measurement of plasma insulin/C-peptide concentrations; (2) 4-hour euglycemic insulin clamp with tritiated glucose, vastus lateralis muscle biopsies (-60 and 240 minutes), and indirect calorimetry; (3) DEXA for percent body fat; (4) 3-3H-glucose turnover before and after starting dapagliflozin to quantitate hepatic glucose production; (5) measurement of insulin signaling (IR/IRS-1 tyrosine phosphorylation and PI-3 kinase activity), mitochondrial function (confocal microscopy), and gene expression (Affymetrix) on muscle biopsies.

Clinical Relevance: Knowledge of how hyperglycemia affects insulin secretion and insulin sensitivity is essential to understanding the pathogenesis of T2DM. Such knowledge will lead to improved therapies for the treatment of patients with T2DM.