Official Study Title: **EFFECT OF FARXIGA ON RENAL FUNCTION AND SIZE IN TYPE 2 DIABETIC PATIENTS WITH HYPERFILTRATION**

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EFFECT OF FARXIGA ON RENAL FUNCTION AND SIZE IN TYPE 2 DIABETIC PATIENTS WITH HYPERFILTRATION

INTRODUCTION

Hyperfiltration is a characteristic feature in experimental models of diabetes and is causally related to an increase in intraglomerular pressure (1-3). In newly diagnosed diabetic patients, both type 1 and type 2, hyperfiltration and enlarged kidney size commonly are observed (4-7), and these hemodynamic/anatomic abnormalities are associated with an increased risk for the development of diabetic nephropathy (4-6).

In poorly controlled diabetic individuals, the filtered load of glucose is markedly increased and glucose – with sodium – reabsorption by the SGLT2 transporter in the proximal tubule is augmented (8-10). As a consequence sodium delivery to the macula densa is reduced (9,10), making the kidney think that it is under perfused and this results in afferent renal arteriolar vasodilation (11). The efferent arteriole of the hyperfiltrating diabetic kidney also is hypersensitive to angiotensin II despite the absence of systemic RAS activation (12,14). The net result of these hemodynamic changes is an increase in intraglomerular pressure and hyperfiltration. Further, angiotensin is a potent growth factor (13,14) and contributes to the increase in size of individual glomeruli and total kidney size (15,16). Since the intraglomerular pressure is related to the radius (r^3) by the Law of LaPlace, the increase in glomerular size also contributes to hyperfiltration (13,14).

Based upon the preceding sequence, it follows that a drug that blocks glucose, along with sodium, reabsorption in the proximal tubule would enhance sodium delivery to the macula densa, cause afferent renal arteriolar constriction, reduce intraglomerular pressure/hyperfiltration, and decrease kidney size. In hyperfiltering diabetic patients with

microalbuminuria, we also would expect the microalbuminuria to decrease. Consistent with this scenario, animal studies have documented that both acute and chronic inhibition of SGLT2 decreases hyperfiltration (16-18) and prevents diabetic nephropathy (19). A recent study in hyperfiltering type 1 diabetic patients treated with empagliflozin has provided additional support for the tubular glomerular feedback hypothesis (11).

We propose to treat newly diagnosed, hyperfiltering T2DM patients with or without microalbuminuria with Farxiga or metformin for 4 months. The metformin-treated group will serve as controls for improved glycemic control, since we have shown that insulin therapy to normalize A1c reduces hyperfiltration and kidney size in T1DM patients (7).

EXPERIMENTAL DESIGN

Subjects: T2D patients who are: (i) drug naïve, (ii) receiving metformin only, (iii) sulfonylurea only; 40 newly diagnosed, drug naïve or on monotherapy (only Metformin or Sulfonylurea), hyperfiltering patients with type 2 diabetes mellitus (T2DM) and 40 newly diagnosed, drug naive or on monotherapy (only Metformin or Sulfonylurea) T2DM patients with normofiltration will participate in the study. Hyperfiltration is defined by GFR $\geq$ 135 ml/min•1.73m^2 and normofiltration by a GFR = 80-134 ml/min•1.73m^2. Other inclusion criteria include: (i) age = 30-70 years; (ii) BMI = 20-45 kg/m^2; (iii) A1c = 7.0% to 12%; (iv) male or female; (v) willingness to participate in the 16 week study protocol; (vi) hematocrit $\geq$ 34%; (vii) GAD antibody negative; (viii) BP < 145/90 mmHg. Exclusion criteria include: (i) > 300 mg/day albumin excretion; (ii) ingestion of medications known to interfere with the renin-angiotensin system or renal function, including diuretic therapy; (iii) hospitalization for unstable angina, history of recent macrovascular (MI/stroke/TIA/ACS) disease, coronary artery revascularization (within 2 months prior to enrollment); (iv) proliferative diabetic retinopathy; (v) history of cancer or major organ system disease; (vi) New York Heart class II-IV heart failure; (vii) severe hepatic insufficiency and/or significant abnormal liver function defined as aspartate aminotransferase

(AST) and/or alanine aminotransferase (ALT) > 3x ULN or total bilirubin > 2.0 mg/dL (34.2 µmo/L); (viii) treatment with steroids, beta blockers, alpha blockers, antiobesity drugs; (ix) pregnant or nursing mothers; (x) premenopausal females who are not practicing acceptable contraceptive methods; (xi) participation in another trial with an investigational drug within 30 days; (xii) alcohol or drug abuse within the preceding 6 months; (xiii) any condition, psychiatric or medical, which in the opinion of the investigator would interfere with the successful completion of the study; (xiv) orthostatic hypotension (≥ 15/10 mmHg decrease upon standing for 3 minutes); (xv) positive serologic evidence of current infectious liver disease including Hepatitis B viral antibody IGM, Hepatitis B surface antigen, Hepatitis C virus antibody and HIV; (xvi) volume depleted patients; (xvii) estimated glomerular filtration rate <60 mL/min•1.73m^2. Patients at risk for volume depletion due to co-existing conditions or concomitant medications, such as loop diuretics should have careful monitoring of their volume status.

Study Protocol

Subjects will have a screening visit to determine eligibility (Visit #1). Prior to or on the day of the screening visit subjects will give their informed, voluntary written consent. During the screening visit, medical history will be taken and physical exam, including weight and height, will be performed. Fasting blood samples will be obtained for routine blood chemistries, CBC, TSH, T4, HbA1c, FPG, , and lipid profile. Urinalysis, urine albumin/creatinine ratio, two 8.5 ml of blood in ACD tubes to assess mitochondrial functions and EKG also will be obtained. The study protocol will be explained to the subject and informed written consent will be obtained. If the subject wishes, he/she will be allowed to take the consent form home to review before signing, as well as complete some of the tests on different days.

Eligible subjects will be asked to collect two 24 hour urines (Visits #2 and #3) for determination of albumin, glucose, sodium, and creatinine excretion. On visit 2, we will draw 17 ml of blood in two ACD tubes to assess mitochondrial functions. On the same day, the two 24

hour urines are collected, plasma creatinine, sodium, and albumin concentrations will be measured.

Within 1-2 weeks, subjects will return to the CRC at 7 AM following an overnight fast (after 9-10 PM) for measurement of renal function (Visit #4) and indirect calorimetry. At 7 AM a catheter is inserted into an antecubital vein and priming doses of inulin (40 mg/kg) and/or iohexol (0.165 X Weight in kg) and para-aminohippurate (8 mg/kg) are given, followed by continuous infusions to maintain plasma concentrations of inulin, iohexol and PAH constant at 20 mg/dl, 100umol/L, and 1.5 mg/dl, respectively. (We have decided to substitute Iohexol for Inulin to assess glomerular filtration rate in this study because the product Inulin is no longer commercially available in the market. Since Inulin cannot be obtained for the purpose originally stated in the study protocol, we have opted to use Iohexol, a compound that determines glomerular filtration rate just as accurately and with the same precision as Inulin). A second catheter will be placed in the contralateral antecubital vein or in a hand vein for withdrawal of blood samples. During the infusion, the subject's hand will be placed in a box heated to 70°C (158°F). Subjects also will ingest a water load (10-15 ml/kg ideal body weight) to ensure spontaneous voiding. Each voided urine will be replaced quantitatively to maintain the water diuresis. After a 90 minute equilibration period for PAH, iohexol, and inulin, subjects will be asked to void and the voided urine will be discarded. Three consecutive urine clearance periods of 30-60 minutes duration will be obtained by spontaneous voiding and plasma inulin, iohexol and PAH concentrations and hematocrit will be measured at the beginning and end of each clearance period. Indirect calorimetry will be performed for 45 minutes (30-75 minutes). Mean arterial blood pressure and heart rate will be continuously monitored by an automated sphygmomanometer over the brachial artery (DINAMAP). At time zero, blood samples will be drawn for HbA1c, FPG, and plasma renin, angiotensin II, aldosterone, FFA , ketones, and NT-pro BNP and 17 ml of blood in two ACD tubes to assess mitochondrial functions, as well as 20 ml of urine. At the end of the study another blood sample will be drawn for FPG, and plasma renin, angiotensin II, aldosterone, FFA , ketones, and NT-pro BNP . Renal size will be measured by magnetic resonance imaging (20).

Following completion of the above studies, subjects who are (i) drug naïve will be randomized to dapagliflozin, 5 mg/day, or metformin-XR, 1000 mg/day. Subjects taking (ii) metformin, will be randomized to dapagliflozin 5 mg/day or glipizide 5 mg/day. Subjects taking (iii) sulfonylurea, will be randomized to dapagliflozin 5 mg/day or metformin-XR, 1000 mg/day. If subjects are on metformin or on a sulfonylurea, the therapy, i.e. metformin or sulfonylurea (including the current dose), will be continued without change. This is an open label study. After 2 weeks (Visit 5), dapagliflozin will be increased to 10 mg/day and metformin-XR to 1000 mg bid; and subjects on glipizide will be increased to 10 mg. Subjects will return for follow up

visits at months 1, 2, and 3 (Visits 6, 7, 8). After 4 months two 24 hour urine collections for albumin, sodium, glucose, and creatinine on visit 9 we also collect 17 ml of blood in two ACD tubes as well as 20 ml of urine to assess the mitochondrial functions. (Visits #9 and #10) will be obtained and the measurement of kidney size (visit #11) and renal blood flow GFR (Visit #12) will be repeated as described above. Subjects will be called every two weeks to ensure compliance with the medication and they will return to the CRC every 4 weeks (Visits #6, #7, and #8) during which time weight, blood pressure, FPG, and HbA1c will be measured and 17 ml of blood in two ACD tubes as well as 20 ml of urine to assess the mitochondrial functions. At study end, i.e. 4 months, HbA1c, FPG and plasma renin, angiotensin II, aldosterone, FFA , ketones, and NT-pro BNP will be measured.

The treatment period could be extended based upon the circumstances (based upon the situation of COVID-19 pandemic) until the repeat studies are completed.

Measurements: Renal plasma flow (RPF) = PAH infusion rate ÷ plasma PAH concentration. Renal blood flow (RBF) = RPF/(1-hct). GFR = $(U_{IN}\ V)/P_{IN}$, Filtration fraction = GFR/RPF. GFR also equals inulin and/or iohexol infusion rate ÷ plasma inulin and/or iohexol concentration. Renal vascular resistance (RVR) = mean arterial pressure ÷ RBF. Kidney size is measured by MRI (20). All renal hemodynamic measurements will be expressed per body surface area (m^2). Plasma renin, angiotensin II, aldosterone, and N-terminal fragment of B-type natriuretic peptide (NT-pro BNP) will be measured by MRI (20).

Statistical Analyses & Expected Results

Based upon the randomization criteria, there will be four groups of type 2 diabetic subjects: hyperfiltering (GFR $\geq$ 135 ml/min•1.73m^2) (GROUP I) and normofiltering (GFR < 135 ml/min•1.73m^2) (GROUP II) treated with dapagliflozin and hyperfiltering (GROUP III) and normofiltering (GROUP IV) diabetic subjects treated with metformin and or a sulfonylurea.

Metformin and sulfonylurea-treated subjects will serve as the control group for improved glycemic control.

The **primary endpoint** is the change from baseline in GFR after treatment with dapagliflozin for 4 months in the hyperfiltering diabetic group (GROUP I) versus the change from baseline in GRF after treatment with metformin for 4 months in the hyperfiltering diabetic group (GROUP III). We expect that dapagliflozin will significantly reduce hyperfiltration compared to baseline and compared to the hyperfiltering diabetic group treated with metformin and/or sulfonylurea . We expect that metformin will have no effect or only a modest effect to reduce hyperfiltration (GROUP III). The change from baseline in GFR in the normofiltering group following 4 months of treatment with dapagliflozin (GROUP II) also will be examined and compared to the change from baseline in GFR in the hyperfiltering group treated with dapagliflozin (GROUP I). We anticipate that dapagliflozin treatment for 4 months will cause a significant decline in GFR in the hyperfiltering group (GROUP I) but will have no effect on GFR in the normofiltering group (GROUP II) treated with dapagliflozin.

Secondary endpoints include changes in renal plasma flow, renal blood flow, renal vascular resistance, and kidney size. In the hyperfiltering diabetic group (GROUP I) treated with dapagliflozin we expect that RPF and RBF will decrease and RVR will rise significantly compared to the hyperfiltering group treated with metformin (GROUP III). We also expect that kidney size will decrease significantly in the hyperfiltering group treated with dapagliflozin (GROUP I) versus the hyperfiltering group treated with metformin (GROUP III). In the normofiltering group (GROUP II) treated with dapagliflozin, we do not expect to observe any significant changes in RPF, RBF, RVR, or kidney size.

Tertiary (exploratory) endpoints include changes in blood pressure, body weight, extracellular fluid volume (inulin space of distribution), plasma renin activity, plasma angiotensin

II, plasma aldosterone, and plasma NT-pro BNP in the 4 treatment groups. We expect that blood pressure and body weight will decrease similarly in hyperfiltering (GROUP I) and normofiltering (GROUP II) diabetic patients treated with dapagliflozin. Further, we anticipate that plasma renin, angiotensin II, and aldosterone levels will increase similarly following dapagliflozin treatment in both GROUPS I and II secondary to the mild intravascular volume.

We expect that urinary albumin excretion (UAE), whether normoalbuminuria or microabluminuria, will decline in the hyperfiltering group treated with dapagliflozin (GROUP I) and remain unchanged in the normofiltering group treated with dapagliflozin (GROUP II).

We anticipate that the HbA1c will decline similarly following dapagliflozin treatment in GROUP I and GROUP II.

As a control for glycemic control, hyperfiltering (GROUP III) and normofiltering (GROUP IV) diabetic subjects will be treated with metformin for 4 months. We expect that the decrease in HbA1c will be similar in metformin-treated (GROUPS III and IV) and dapagliflozin-treated (GROUPS I and II) groups. Despite the similar reduction in HbA1c in metformin-treated and dapagliflozin-treated subjects, we do not expect that metformin treatment (in GROUP III and GROUP IV) will have any effect on RBF, RPF, RVR, kidney size, plasma renin/angiotensin II/aldosterone levels, NT-pro BNP, or urinary albumin excretion.

SAMPLE SIZE CALCULATIONS:

The sample size was calculated to evaluate the primary endpoint (change in GFR from baseline) between the hyperfiltering group treated with dapagliflozin (GROUP I) and the hyperfiltering group treated with metformin (GROUP III). Assuming that dapagliflozin reduces the GRF by -33±24 ml/min•1.73m^2 (172±23 to 139±25) and metformin has no significant effect on GRF (0±13; 117±11 to 117±15) (11), we calculated that a sample size of 18 per group would provide 90% power at alpha = 0.01 to detect a mean GFR difference from baseline of 33

ml/min•1.73m^2 in the dapagliflozin-treated versus metformin-treated groups (GROUP I versus GROUP III). The sample size (n =18) would have similar power to detect a mean GFR difference from baseline of 33 ml/min•1.73m^2 between the dapagliflozin-treated hyperfiltering group (GROUP I) versus the dapagliflozin-treated nonhyperfiltering group (GROUP II) [PASS Version 11, NCSS Kaysville Utah 2011]. To accommodate 10% loss to follow-up we will enroll 20 (=18/0.9) subjects per group x 4 groups = 80 subjects.

Based upon published data (11), we anticipate that a sample size of 18 also will be sufficient to provide significant within and between group differences for changes in RBF, RPF, and RVR between the dapagliflozin-treated hyperfiltering group (GROUP I) and the metformin-treated hyperfiltering group (GROUP III).

STATISTICAL METHODS: Continuously distributed outcomes will be summarized with the sample size, mean, standard deviation, median, minimum and maximum and categorical outcomes will be summarized with frequencies and percentages. The significance of treatment group contrasts with respect to mean changes in GFR will be assessed with analyses of variance on changes from baseline with a Tukey correction for multiple comparisons. Changes in renal plasma flow, renal blood flow, renal vascular resistance, and kidney size will be analyzed similarly. Within group assessments of the significance of changes in the mean from baseline will be carried out with paired t-tests. The significance of group contrasts on binary and categorical outcomes will be assessed with Fisher's exact test; assessments of within-group changes from baseline will be carried with McNemar's test. If safety data are available, treatment groups will be contrasted with regard to the occurrence of adverse events with Fisher's Exact test. Continuously distributed outcomes may be log transformed to approximate normality prior to analysis. All statistical testing will be two-sided with a nominal significance level of 5%. SAS Version 9.3 and R will be used throughout. Formatted tables and graphics will be prepared in Microsoft Word directly from the database using a series of SAS and R

scripts. All code, databases, and output will be delivered to the sponsor at the conclusion of the study.

RESCUE THERAPY: Only newly diagnosed, drug naïve or on monotherapy (metformin or sulfonylurea) type 2 diabetic subjects with a HbA1c = 7.5-12.0% will be eligible. Therefore, we anticipate that these individuals will have a good response to both dapagliflozin, metformin, and glipizide therapy. If after 2 months the fasting plasma glucose concentration remains above 170 mg/dl, the subject will be discontinued from the study and started on an antidiabetic regimen as deemed appropriate by the primary care physician. All subjects who are discontinued will be replaced.

Safety: The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section and is properly trained.

Definition of adverse events: An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an adverse event can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered. The term adverse event is used to include both serious and non-serious adverse events. If a participant becomes pregnant, she will be asked to stop all study medication and sign the pregnancy consent form. While being asked to sign the form, she will be taking part in the follow-up, her personal information related only to her pregnancy and outcome will be collected and reviewed to better understand the effects of dapagliflozin (Farxiga) on her pregnancy and the fetus/child.

Definitions of serious adverse event: A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

The causality of serious adverse events (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca and the Institutional Review Board.

Recording of adverse events

Time period for collection of adverse events: It is the responsibility of the investigator to perform periodic and special assessments for adverse events. All adverse events will be summarized and reported annually to the Institutional Review Board. The investigator and research staff will note all AEs offered by the subject after administration of either glipizide, metformin and/or dapagliflozin or placebo. All clinical complaints volunteered by, or elicited from, the subject during the study will be recorded in the appropriate section of the case report form for the study period indicated. AEs may include events that occur as a result of protocol-

mandated procedures (e.g., events due to invasive procedures). If any AE occurs, the subject will receive appropriate treatment and medical supervision and will be followed until the AE is stabilized or to a point where it is no longer clinically significant. As previously noted, all medical conditions and abnormal findings present before administration of the first dose of glipizide, metformin or dapagliflozin or placebo will be recorded as concurrent illnesses or baseline symptoms.

Follow-up of unresolved adverse events: All AEs judged to be clinically significant, including clinically significant laboratory abnormalities, will be followed until resolution. The following variables will be collected for each AE:

- AE (verbatim)
- The date and time when the AE started and stopped
- Whether the AE is serious or not (that is, the intensity – mild, moderate or severe)
- Investigator causality rating against the Investigational Product or procedures (unlikely, possibly, probably, definitely)
- Action taken with regard to investigational product/procedure
- Adverse event caused subject's withdrawal from study (yes or no)
- Outcome (recovered, recovering, ongoing, fatal – see SAE).

In addition, the following variables will be collected for serious adverse events:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to:

- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Causality assessment in relation to other medication
- Causality assessment in relation to additional study drug
- Description of AE.
- Date reported to IRB

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in the section titled: “Definitions of Serious Adverse Events”. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Adverse Events based on signs and symptoms: When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if

a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests: Deterioration as compared to baseline in protocol-mandated glucose values and/or blood pressure values should therefore only be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in glucose concentration and/or blood pressure is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Reporting of serious adverse events:

All SAEs have to be reported, whether or not considered causally related to the IP, or to the study procedure(s). All SAEs will be recorded in the source document and reported annually to the UTHSCSA IRB during continuing review.

Serious adverse events that do not require expedited reporting to the FDA need to be reported to AstraZeneca at least quarterly preferably using the MedDRA coding language for serious adverse events.

Investigator will review all SAEs and determine whether the reviewed incident, experience, and outcome represents a possible UPIRSO (Unanticipated Problem Involving Risk To Subjects Or Others). All possible UPIRSOs will be reported to the UTHSCSA IRB using the Prompt Reporting Form.

a) Prompt reporting timeframe - report is made to the IRB within 7 days for UPIRSOs based on internal information (e.g., experienced by subjects enrolled by the investigator(s) at an institution affiliated with the UTHSCSA IRB) or 14 days for UPIRSOs based on external information (e.g., experienced by subjects enrolled by the investigator(s) at an institution not affiliated with the UTHSCSA IRB)

b) Special shortened reporting timeframe: All UPIRSOs based on internal information that are either life threatening or fatal events must be reported within 48 hours.

Investigators and other site personnel must inform the FDA, via a MedWatch/AdEERs form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to AstraZeneca. A copy of the MedWatch/AdEERs report must be faxed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

When reporting to AstraZeneca, a cover page should accompany the MedWatch/AdEERs form indicating the following:

- Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA

- The investigator's name and address
- The trial name/title and AstraZeneca ISS reference number

Investigative site must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

Send SAE report and accompanying cover page by way of fax to AstraZeneca's designated fax line: 1-866-984-7229 or email to AEMailboxClinicalTrialTCS@astrazeneca.com

Serious adverse events that do not require expedited reporting to the FDA need to be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events.

In the case of blinded trials, AstraZeneca will request that the Sponsor either provide a copy of the randomization code/ code break information or unblind those SAEs which require expedited reporting.

All SAEs have to be reported to AstraZeneca, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the regulatory authority (FDA) of the SAE as per local requirements.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. The investigators or other site personnel will inform the UTHSCSA IRB of any follow-up information on a previously reported UPIRSO within 2 calendar days i.e., immediately but no later than 48 hours of when he or she becomes aware of it.

SAFETY MONITORING BOARD

Two independent physician-scientists associated with the UTHSCSA (Dr.Deivjit Tripathy and Dr. Nicholas Musi) will comprise the safety monitoring board for this study. They will meet every 6

months and review the adverse events and all potential risks arising from the study, study drugs and procedures. They will report to the Principal Investigator any and all findings deemed serious and, which would require a protocol revision or study termination. As necessary, a specific report also will be forwarded to the UT-IRB and to all sites involved in the study.

INVESTIGATORS/SITES

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Aim:

To explore the changes in mitochondrial function from baseline as a prominent pathway in the dynamics of glomerular filtration rate in hyperfiltrating groups (Group I and Group III).

Justification:

Mitochondria are multivariate signal processors which convert macromolecular energy sources into a chemical and physical energies ATP and heat respectively. Aberrations of mitochondrial function could be resulting in type 2 diabetes. Since mitochondria are dynamic organelles and exist as heterogeneity in human tissues, the phenotypic spectrum of mitochondrial disorders can present either in infancy or adulthood, and in multisystemic or highly tissue-specific manner. Although several signature traits appear in patients, the functional assessment of mitochondria in normal and disease states are huge challenges for medical treatments. Our ability to define the genetic and environmental bases of these disorders has accelerated and new questions have arisen. Why are some diseases highly tissue-specific, and other multisystemic? We are all puzzled now why some drugs that inhibit mitochondria cause disease, while other modulators are protective against it? The answers to most of these questions are a mystery, but if identified these could provide fundamental insight into metabolism, lead to new therapies that are associated with mitochondrial dysfunction.

Study protocol:

In the past, we have developed several cutting-edge technologies to assess mitochondrial functional changes in normal and disease models in vitro. An exciting new frontier in mitochondrial medicine is defining the regulatory mechanisms that give rise to the observed heterogeneity nature of mitochondria within cells and tissues. We will be developing tools to better understand the mitochondrial biogenesis, movement, dynamics, shape, and quality control. For instance, mitochondrial mass and volume will be determined using volume scope (serial block-face scanning electron microscopy). Our mission is to explore cutting-edge optical imaging-based methods to address major questions pertaining to mitochondrial signaling and cellular function in situ. To establish the assessment of mitochondrial bioenergetics parameters (morphology, membrane potential, NADH, ROS, Redox state, ATP, Ca2+) in leukocytes and platelets, we will image mitochondrial dynamics and function using live-confocal imaging. Although genetic tools are available to monitor mitochondrial nature in cellular milieu, we will

explore and identify the endogenous molecules that holds inherent fluorescent properties like NAD(P)H, metabolites, lipids, proteins. To accomplish these tasks, we will acquire intra-vital multiphoton imaging system which will have an ability to tune wide-range of visualization capability. The anticipated outcome of these approaches will significantly enhance our knowledge on the treatment outcomes and patient care.

Platelets in circulating blood contain certain amounts of fully functional mitochondria, which have been used as a valuable tool for mitochondrial function research owing to their easy accessibility compared with other metabolically active tissues. The rapid development of understanding in platelet mitochondria will provide us a unique way to translate its function into knowledge on human health and disease. Future efforts in this direction are highly needed for extending our knowledge to the establishment of potential clinical biomarkers as well as precision medicine for diabetic related metabolic diseases. Although fewer studies have conducted some mitochondrial functional assessments, we are in a better position to assess multiple mitochondrial bioenergetics parameters, sensing as well as shape transition during human health style, disease progression, and treatment outcomes. Similarly, we will also utilize buffy coat (WBCs) to examine mitochondrial bienergetic parameters and mitochondrial DNA copy number and redox changes.