CLINICAL TRIAL PROTOCOL

PROTOCOL TITLE: Metabolic Effects of the SGLT-2 Inhibitor Empagliflozin in Patients with Diabetic Nephropathy (MEDiaN)

PROTOCOL NUMBER: MEDiaN2018

PROTOCOL VERSION: V1.3
PROTOCOL DATE: 9 December 2018

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PROTOCOL SIGNATURE PAGE

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Funding body: Tanoto Initiative For Diabetes Research Award (Duke-NUS-TIDR/2018/0010)

Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described trial in compliance with all stipulations of the protocol, regulations and ICH E6 Guideline for Good Clinical Practice (GCP)

Principal Investigator Name: LAM YUN RUI AMANDA

Principal Investigator Signature: _____

Date: _____

1 BACKGROUND AND RATIONALE

Diabetic nephropathy (DN) is a common cause of end-stage renal disease (ESRD) and accounts for nearly half of all new patients starting dialysis in Singapore, the country with the highest rates of DN in the Asia-Pacific region. Despite the scale of the problem, little progress has been made in our understanding of the pathogenesis of the disorder and no new therapies have been offered.

Dysregulated fuel metabolism as a major driver of diabetic nephropathy

We have conducted a metabolomics study of human diabetic nephropathy that revealed evidence for alterations in mitochondrial fuel metabolism in patients with the disease, a finding also reported in other recent studies of human DN[1,2]. This study, which was conducted in collaboration with clinician-scientists at Khoo Teck Puat Hospital (KTPH) in Singapore, was a comprehensive metabolic profiling study of over 300 human diabetic nephropathy patients using our tandem MS-based metabolomics platforms. The study used serum from patients with diabetic nephropathy and matched controls with diabetes but without nephropathy. The patients with diabetic nephropathy showed a metabolite pattern suggesting alterations in fatty acid oxidation (Figure 1).

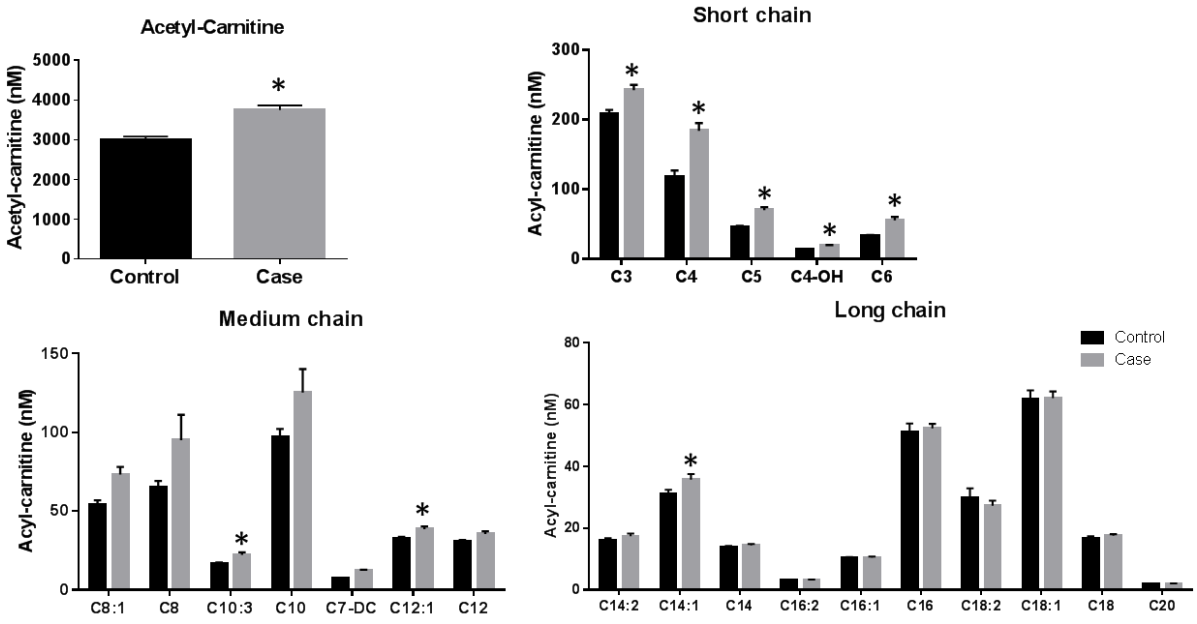

Figure 1: Acyl-carnitine species in human subjects with diabetic nephropathy are elevated. The serum acyl-carnitine profile of subjects with type 2 diabetes and urine albumin-creatinine ratio >300 (n=149) was compared to the profile from matched controls (n=150) with diabetes of equal duration but no evidence of proteinuria. *p<0.05.

The metabolomics profiling study showed that patients with DN have elevated levels of several short chain acyl-carnitines (Figure 1, top right) including C3 (propionyl-), C4 (butyryl-) and C5 (isovaleryl-carnitine). This pattern of elevated short-chain acyl-carnitines resembles what is observed in several inherited metabolic disorders including short-chain acyl-coA dehydrogenase (SCAD).

A similar analysis of urine from patients with DN demonstrate alterations in the abundance of several TCA-cycle related organic acids (JJ Liu et al, manuscript under review).

Based on this we hypothesize that **dysregulated mitochondrial fuel oxidation is a major driver of**

diabetic nephropathy.

Renal glucose handling and the SGLT-2 inhibitors

The **SGLT-2 inhibitor** (SGLT-2i) class of drugs is a recently approved treatment for diabetes, which lower blood glucose levels by inhibiting renal reabsorption of glucose by SGLT-2 transporters in the proximal renal tubule, resulting in glucosuria[3].

SGLT-2i and Diabetes Complications

Clinical trials of SGLT-2i in patients with type 2 diabetes (T2DM) have shown unexpectedly strong effects of the drug on diabetes complications[4,5]. In the EMPA-REG OUTCOME trial, the SGLT-2i empagliflozin was able to i) reduce the risk of cardiovascular death and ii) slow the progression of DN in patients with diabetes. The effect of the drug on cardiac and kidney complications seems to be out of proportion to the modest glucose lowering ability of the drug. The reason for the protective effect on cardiac and kidney complications remains unexplained.

SGLT-2i and Fuel Metabolism

Attempts to explain how SGLT-2i protect against diabetes complications have focused on several aspects of the drug's action including the effect on whole body fuel metabolism[6–8]. The forced excretion of glucose in the urine leads to unique physiologic changes. Patients on the drug have lower glucose and insulin levels and raised glucagon. There is reduced glucose use, increased lipolysis with a resultant rise in fatty acid oxidation and ketogenesis[9]. Interestingly, recent studies have also reported a rise in activity of the urea cycle[10]. The net effect is a major shift in fuel metabolism away from glucose towards the burning of fat and ketone bodies. The shift in fuel metabolism is of particular interest given our recent findings of altered fuel metabolism in patients with diabetic nephropathy.

Study aims

The aim of our proposed study is to **examine the state of fuel metabolism in patients with DN both before and after the use of SGLT-2i**. We anticipate that 30 days of treatment with the SGLT-2i empagliflozin will induce a shift in fuel metabolism in subjects with DN.

Our hypothesis is that dysregulated fuel metabolism is a major driver of diabetic nephropathy. We propose that the beneficial effect of SGLT-2i in DN is related to changes in fuel metabolism. Our anticipated outcomes include reversal of many of the metabolomics findings reported in DN. We expect decreased fasting glucose and elevated serum ketones, as previously reported. We also predict reductions in the levels of short-chain acyl-carnitines, increased C4-OH and long-chain acyl-carnitines as well as reductions in urinary organic acid excretion. Unfortunately, the scope of this proposal is too short to expect significant changes in albuminuria, although this will be assessed. If completed successfully, this project will help to link changes in mitochondrial fuel metabolism with the beneficial effects of the SGLT-2i class of drugs.

Our goals are to better understand the contribution of fuel metabolism to the development of DN, and to determine if changes to fuel metabolism can have a positive impact on this disease.

1.1 General Introduction

Empagliflozin is a SGLT-2 inhibitor which is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus, and to reduce the risk of cardiovascular death in adult patients with type 2 diabetes mellitus and established cardiovascular disease. By inhibiting sodium-glucose co-transporter 2 (SGLT2), the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation, empagliflozin reduces renal reabsorption of filtered glucose, thereby increasing urinary glucose excretion. Presently empagliflozin is not indicated for the treatment of diabetic nephropathy (DN), although it has been shown in previous studies to have a beneficial effect on DN.

1.2 Rationale and Justification for the Study

We hypothesize that treatment with empagliflozin taken orally at 10mg per day for 30 days will lead to significant changes in the circulating metabolomics pattern in patients with DN.

Since we are interested in understanding the metabolic effects of the drug, we propose to administer the drug for a period of 30 days. We will perform a comprehensive analysis of the state of fuel metabolism in these patients before, during and after the administration of empagliflozin using targeted metabolomics and other approaches. Our goal is to discover key changes in fuel metabolism in DN patients receiving empagliflozin.

1.2.1 Rationale for the Study Purpose

Clinical trials of SGLT-2i in patients with type 2 diabetes (T2DM) have shown unexpectedly strong effects of the drug on diabetes complications, including slowing the progression of DN. The mechanisms underlying the protective effect on renal complications of diabetes have not been fully elucidated.

Existing literature suggest that the forced excretion of glucose in the urine resulting from SGLT-2i use leads to unique physiologic changes, leading to a major shift in fuel metabolism away from glucose towards the burning of fat and ketone bodies. The shift in fuel metabolism is of particular interest given our recent findings of altered fuel metabolism in patients with diabetic nephropathy.

The aim of our study is to examine the state of fuel metabolism in patients with DN both before and after the use of SGLT-2i. If completed successfully, this study will help to link changes in mitochondrial fuel metabolism with the beneficial effects of the SGLT-2i class of drugs. A better understanding of the relationship between mitochondrial fuel metabolism, DN, and the metabolomic effects of SGLT-2i may point a way to more therapies for DN which target mitochondrial fuel metabolism.

1.2.2 Rationale for Doses Selected

The dose of empagliflozin 10mg per day is selected as it is the recommended initiation dose according to the prescribing information provided by the manufacturers. The dose selection is similar to dosages used in patients in standard clinical practice and in large clinical trials.

1.2.3 Rationale for Study Population

DN is the most common cause of end-stage renal disease (ESRD) in the US and also accounts for nearly

half of all new patients starting dialysis in Singapore, the highest in the Asia-Pacific region. ESRD is also a major cause of death, accounting for over 65% of deaths in T1DM patients with nephropathy and the 5-year survival rate being typically less than 10% in elderly patients with T2DM. Despite these sobering statistics, little progress has been made in early diagnostic markers or the availability of increased treatment options for the clinical management of DN. Current mainstay of therapy relies on control of hyperglycemia and blood pressure and pharmacologic blockade of the renin-angiotensin system (RAS) with ACE inhibitors or angiotensin receptor blockers (ARBs). Despite these treatments, DN typically progresses over time. Finally, no new therapies for DN have been offered the past few years and to our knowledge, there are only a couple of ongoing clinical trials in the US for potential new treatments for DN (clinicaltrials.gov).

1.2.4 Rationale for Study Design

We will conduct a single-arm open-label intervention study to examine the effects of a standard dose of empagliflozin on fuel oxidation patterns in patients with type 2 diabetes and DN. No randomization or blinding will be performed as the response to empagliflozin is stereotypical and the measured parameters are objective and thus not subject to observer bias.

2 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

Our study hypothesis is that dysregulated mitochondrial fuel oxidation, as measured by changes in the circulating metabolomics pattern of DN patients, is a major driver of diabetic nephropathy. Furthermore, we hypothesize that treatment with empagliflozin over a period of 30 days will reverse the metabolomics changes seen in patients with DN.

2.2 Primary Objectives

The primary objective is to study the effects of empagliflozin on fuel metabolism patterns in patients with DN. We will conduct a single arm intervention study to examine the effects of a standard dose of empagliflozin on fuel oxidation patterns in patients with type 2 diabetes and DN. Since we are interested in understanding the metabolic effects of the drug, we propose to administer the drug for a period of 30 days. We will perform a comprehensive analysis of the state of fuel metabolism in these patients before and after the administration of empagliflozin using targeted metabolomics and other approaches. Our goal is to discover key changes in fuel metabolism in DN patients receiving empagliflozin.

2.3 Secondary Objectives

N.A.

2.4 Potential Risks and Benefits:

2.4.1 Potential Risks

Venepuncture

This study requires blood to be drawn from a vein in the arm. For most people, blood draws using needle punctures do not cause any serious problems. However, drawing blood may result in pain at the point of

puncture, a feeling of faintness, irritation of the vein and bruising or bleeding at the site of needle puncture. There is also a very slight possibility of an infection at needle puncture site. An experienced staff will perform these procedures and every precaution will be taken to prevent infection.

Indirect calorimetry

Indirect calorimetry involves the placement of a transparent plastic hood with ventilation over the head for 30-minute intervals during the 2nd and 3rd study visit. It is well tolerated but some people may feel claustrophobic. To avoid this, a brief trial run will be performed for subjects to familiarize with the equipment and measurement.

Adverse Effects Related to empagliflozin

A) Allergic Reactions

All drugs have a potential risk of an allergic reaction. Allergic reactions range from mild reactions such as skin itchiness, rash, eye swelling to more severe allergic reactions that affects the blood pressure (allergic anaphylaxis). Severe allergic reactions could become life threating if not treated quickly.

B) Hypotension

Empagliflozin causes intravascular volume contraction. The incidence of symptomatic hypotension is greater in patients with renal impairment, elderly individuals, in patients with low systolic blood pressure, and patients on diuretics. Subjects will be advised to avoid prolonged periods of fasting or water deprivation. They will be advised to maintain an adequate intake of water especially during periods of greater insensible fluid loss (eg: exercise or hot weather). All subjects will be educated about signs and symptoms of hypotension. Individuals above age 65 years, or with more severe renal impairment will be excluded from this study.

C) Ketoacidosis

Reports of ketoacidosis, a serious condition requiring urgent hospitalization have been reported in patients taking empagliflozin. Some of these cases were identified in patients with type 1 diabetes, in whom empagliflozin is not indicated. We have excluded patients with type 1 diabetes from our study. In patients with T2DM receiving empagliflozin, the FDA identified potential triggering factors for ketoacidosis including intercurrent illness, reduced food and fluid intake due to illness or surgery, reduced insulin doses, history of alcohol intake, and pancreatic disorders suggesting insulin deficiency (eg: type 1 diabetes, history of pancreatitis, pancreatic surgery). All subjects will be assessed by a physician for factors that may predispose to ketoacidosis, including pancreatic insulin deficiency from any cause, caloric restriction, and alcohol abuse. Subjects in whom these factors are identified will be excluded from the study. All subjects will be educated to temporarily discontinue empagliflozin in situations known to predispose to ketoacidosis (eg: intercurrent illness, prolonged fasting due to acute illness or surgery), and how to identify symptoms suggestive of ketoacidosis and to seek medical attention. Subjects may contact the study team should they fall ill. The study team will advise patients regarding period of discontinuation of study drug, if applicable.

D) Acute kidney injury and impairment in renal function

Treatment with empagliflozin is known to cause an initial mild fall in eGFR which is related to one of its underlying mechanism of actions in altering tubuloglomerular feedback. This initial mild fall in eGFR is followed by a progressive recovery of GFR during the following weeks. Empagliflozin may also result in acute kidney injury or significantly increased serum creatinine as a result of intravascular volume contraction. Factors that may predispose to acute kidney injury include more severe renal impairment,

congestive heart failure, and some concomitant medications (including diuretics, ACE inhibitors, angiotensin receptor blockers, and NSAIDs). Subjects with more severe renal impairment will be excluded from this study. Subjects will be educated on situations that predispose to acute kidney injury, such as reduced oral intake or increased fluid losses, and to temporarily discontinue empagliflozin if these occur. All subjects will have their kidney function tested before and monitored during this study.

E) Genital mycotic infections, urinary tract infections, urosepsis and pyelonephritis
Treatment with SGLT-2i increases the risk for genital mycotic infections and urinary tract infections, which are usually mild. Serious urinary tract infections including urosepsis and pyelonephritis requiring hospitalization in patients receiving SGLT-2i have been described. Patients with a history of recurrent urinary tract or genital mycotic infections, and patients with a history of serious urinary tract infections will be excluded from this study.

F) Fournier's gangrene
On 29th August 2018, the U.S. Food and Drug Administration (FDA) warned that cases of a rare but serious infection of the genitals and area around the genitals have been reported with SGLT2 inhibitors. This serious rare infection, called necrotizing fasciitis of the perineum, is also referred to as Fournier's gangrene. In the five years from March 2013 to May 2018, 12 cases of Fournier's gangrene were identified in patients taking an SGLT2 inhibitor (7 men and 5 women), all of whom were hospitalized and required surgery. In 2017, an estimated 1.7 million patients received a dispensed prescription for an SGLT2 inhibitor from U.S. outpatient retail pharmacies. Fournier's gangrene developed within several months of the patients starting an SGLT2 inhibitor. In accordance with recommendations made by the U.S. FDA, patients will be counselled regarding the risk of Fournier's gangrene before commencing treatment with empagliflozin. Patients will be counselled to seek medical attention immediately if they experience any symptoms of tenderness, redness, or swelling of the genitals or the area from the genitals back to the rectum, and have a fever above 38.0 degrees Celsius, or a general feeling of being unwell.

G) Hypoglycemia
Treatment with empagliflozin is expected to result in a fall in blood glucose. Hypoglycemia is a potential side effect of all glucose-lowering medications, including empagliflozin. Patients who are at high risk of significant hypoglycaemia will be excluded from the study. Patients at high risk of significant hypoglycaemia include those with 1 or more episodes of severe hypoglycaemia in the last 1 year, and frequent (at least once a week) hypoglycaemia episodes of any severity.

Issues Related to Pregnancy and Breast-Feeding
There is inadequate data about the use of empagliflozin in pregnant or breast-feeding women. Therefore, pregnant or breast-feeding women cannot participate in this study. Sexually active women of childbearing potential must use a reliable method of birth control while participating in this study. Reliable methods of birth control are considered to be abstinence (not having sex), oral contraceptives (the pill), intrauterine device (IUD), DepoProvera, Norplant, tubal ligation (tubes tied) or vasectomy of the partner. An acceptable although less reliable method, involved the careful use of condoms and/or a spermicidal foam or gel along with a diaphragm, cervical cap, or sponge. Study subjects would be encouraged to discuss this issue further with their doctor if they any questions.

Issues Related to Pediatric Use
The safety and effectiveness of empagliflozin in pediatric patients under 18 years of age have not been established. Patients under 21 years of age will be excluded from this study.

2.4.2 Potential Benefits

There is unlikely any direct benefit from participating in this study as treatment with empagliflozin for one month is unlikely to provide any long-term benefit. However, completion of the study will provide us with a better understanding of the relationship between empagliflozin use and mitochondrial fuel oxidation, and the biochemical changes surrounding the development of DN. The results of the study may point a way to more therapies for DN which target mitochondrial fuel metabolism.

3 STUDY POPULATION

3.1 List The Number and Nature of Subjects to be Enrolled.

Assuming that treatment with empagliflozin would result in a significant shift in metabolic fuel selection, 40 subjects with DN will be required. Assuming a screen failure rate of 1 in 3, a total of 60 subjects will need to be recruited from the Singapore General Hospital (SGH) Diabetes and Metabolism Centre (DMC) clinics. There are no subject restrictions based on race of the subject in this study.

3.2 Criteria for Recruitment and Recruitment Process

This will be a single-centre study conducted in SGH. Subjects from this trial will be patients attending the Diabetes and Metabolism Centre (DMC) clinics. Physicians at the DMC can refer any interested and suitable patients to the study team.

The study team will provide additional details regarding this study to all interested patients and they will be scheduled for consent taking and screening visit at the SGH Clinical Trials Research Centre (CTRC).

3.3 Inclusion Criteria

Subject must meet all of the inclusion criteria listed below to participate in this study:

1. Man or woman between 21 and 65 years of age

2. Type 2 diabetes mellitus as defined by:
   - Fasting plasma glucose ≥7.0mmol/l, or
   - Symptoms of hyperglycemia with casual plasma glucose ≥11.1 mmol/L, or
   - 2-hour plasma glucose ≥11.1 mmol/l after a 75-gram oral glucose load, or
   - Known type 2 diabetes mellitus diagnosed by a medical practitioner

3. Two or more measurements indicating increased urine protein excretion within 1-year

   Increased urine protein excretion is defined as:
   - Urine microalbumin/creatinine ratio (ACR) > 3.3 mg/mmol creatinine or
   - Urine total protein/creatinine ratio (PCR) > 0.2 g/urine creatinine

4. Known diabetes duration > 3 months

5. HbA1c 7.5 – 9% (within 3 months prior to enrolment)

6. Not currently treated with an SGLT-2 inhibitor, and have not received SGLT-2 inhibitor therapy within the last 10 weeks.

7. Stable diabetes therapy for at least 3months as defined as:
   - No change in dose of diabetes medications by more than two-fold or
   - No new agents added within the previous 3 months

8. Stable doses of angiotensin converting enzyme (ACE) inhibitors or angiotensin AT(1)-receptor blockers (ARBs) for at least 3 months.

9. Capable of providing informed consent

Subjects with HbA1C between 7.5 – 9% will be recruited because treatment with SGLT-2i is indicated in these patients according to local clinical practice guidelines. Subjects with HbA1C > 9% will be excluded due to safety considerations from uncontrolled hyperglycemia. We will exclude subjects on insulin treatment as insulin treatment may interfere with measured parameters.

We will not be studying children, or adults above the age of 65. The risk of drug related adverse effects increases in the elderly. All methods of contraception are allowed in women of childbearing age.

3.4 Exclusion Criteria

Exclusion criteria were selected to enhance safety and adherence. All subjects meeting any of the exclusion criteria at baseline will be excluded from participation.

1. Type 1 diabetes mellitus
2. Insulin treatment
3. Ketosis-prone diabetes
4. Previous diabetic ketoacidosis

5. Known peripheral vascular disease or history of lower limb amputations
6. History of Fournier's gangrene or skin and soft tissue infections of the perineum
7. Recurrent or severe urinary tract or genital mycotic infections, or history of genitourinary infection within 2 weeks prior to informed consent
8. Known allergic reaction to empagliflozin or other SGLT2 inhibitor
9. Significant renal impairment (estimated Glomerular Filtration Rate < 45 ml/min/1.73m2**)
10. Dialysis or kidney transplant
11. Renal artery stenosis
12. Alanine aminotransferase or aspartate aminotransferase above 3x upper limit of normal
13. Significant change in weight (≥10% in the preceding 6 months)
14. Treatment with anti-obesity drugs
15. Previous bariatric surgery or other gastrointestinal surgeries that induce chronic malabsorption
16. Treatment with systemic glucocorticoids
17. Blood dyscrasias or clinically significant anaemia (Haemoglobin < 10 g/L)
18. Cancer within the last 5 years (except basal cell carcinoma)
19. Medical condition likely to limit survival to less than 3 years
20. Uncontrolled thyrotoxicosis, untreated hypothyroidism
21. Any ongoing acute medical illnesses
22. Hospitalization within 1 month prior to enrolment
23. Nursing mothers
24. Pregnancy, currently trying to become pregnant, or of child-bearing potential and not practicing an acceptable method of birth control or do not plan to continue using this method throughout the study
25. Excessive alcohol intake (> 1 unit per day for women and > 2 units per day for men)
26. History of drug abuse
27. Pancreatic insulin deficiency from any cause (history of pancreatitis, pancreatic surgery)
28. Known intolerance or allergic reactions to empagliflozin or other SGLT-2 inhibitors
29. Current participation in another clinical trial, or ingestion of investigational drug in another trial within 30 days prior to enrolment.
30. Chronic liver disease:
    - Hepatitis B
    - Hepatitis C
    - Autoimmune hepatitis
    - Hemochromatosis
31. Presence of any non-DN renal glomerular disease
    (e.g. IgA nephropathy, lupus nephritis, membranous glomerulonephritis, focal segmental glomerular sclerosis)
32. Any previous organ transplantation
33. Any factors likely to limit adherence to interventions (e.g. dementia; alcohol or substance abuse; history of unreliability in medication taking or appointment keeping; significant concerns about participation in the study from spouse, significant other or family members)
34. Failure to obtain informed consent from participant

**MDRD formula: eGFR (ml/min) = 175 x [Screatinine (umol/L)/88.4]$^{-1.154}$ x [age]$^{-0.203}$ x 0.742 [if patient is female]
Ann Intern Med. 1999 Mar 16;130(6):461-70. Am J Kidney Dis. 2002 Feb;39(2 Suppl 1):S1-266.

3.5 Subject Replacement

There will be no replacement of subjects who drop out of the study.

4 STUDY DESIGN

In this single-centre, open-label study, 40 adults with DN will be recruited over 2 years. Following screening and baseline metabolic evaluations, eligible subjects will be treated with empagliflozin for 30-days and re-assessed.

4.1 Randomisation and Blinding

No randomization or blinding will be performed as the response to empagliflozin is stereotypical and the measured parameters are objective and thus not subject to observer bias.

4.2 Contraception and Pregnancy Testing

There are no adequate data from the use of empagliflozin in pregnant woman. Therefore, pregnant women cannot participate in this study. Female subjects of childbearing potential will be tested for pregnancy using urine pregnancy test kits during their screening (**Visit 1**) and dosing visits **(Visit 2).** Sexually active women of childbearing potential must use a reliable method of birth control while participating in this study. Reliable methods of birth control are considered to be abstinence (not having sex), oral contraceptives (the pill), intrauterine device (IUD), DepoProvera, Norplant, tubal ligation (tubes tied) or vasectomy of the partner. An acceptable although less reliable method, involved the careful use of condoms and/or a spermicidal foam or gel along with a diaphragm, cervical cap, or sponge. Study subjects would be encouraged to discuss this issue further with their doctor if they have any questions.

4.3 Study Visits and Procedures

4.3.1 Patients who meet inclusion criteria and do not meet any of the exclusion criteria will be invited to participate during their visit to the DMC at SGH by the PI, Co-Is or their managing physician. Interested patients will be scheduled for their first study visit **(Visit 1) within 28 days** at SGH CTRC.

4.3.2 VISIT 1: Informed Consent and Screening

Written informed consent will be taken by the principal investigator, co-investigators or study team members assigned by the PI. After consent has been taken, study procedures in **Table 1** will be performed. The PI, Co-I or study team member(s) assigned by the PI will review screening results to determine whether subjects are eligible to continue with the study. Information regarding all concomitant therapy taken by the patients will be collected.

Table 1. Study Procedures

	Visit 1	Visit 2	Visit 3
	Consent & Screening	Dosing & Metabolic Evaluation	Post-treatment and Final Visit
Administrative			
Informed consent	X		
Inclusion/Exclusion criteria	X		
Dispense Medication		X	
Monitoring of drug compliance			X
Clinical Procedures/Assessment			
Fasting (≥8 hours)		X	X
Medical History/Assessment	X		X
Drug History	X		X
Adverse Effects Monitoring			X
General Physical Examination	X		X
BP/Pulse	X		X
Weight (triplicate) *	X		X
Height (triplicate) *	X		
Hip/Waist Circumference (triplicate) **	X		X
Indirect Calorimetry ***		X	X
Laboratory Procedures			
HbA1C	X		
Fasting Glucose		X	X
Full Blood Count	X		
Potassium	X		X

Creatinine	X		X
eGFR Calculation	X		X
Lipid Panel		X	X
Urine βHCG (female of child bearing potential)	X	X	
ALT	X		X
AST	X		X
Metabolomic Profiling		X	X
Urine ACR	X		X
Urine PCR		X	X

* Weight will be measured in light clothing to the nearest 0.1 kg on a balance scale and height with a stadiometer three times and then averaged

** Waist circumference will be measured from the inferior margin of the last rib and hip circumference measured at the crest of the ileum to the nearest 0.1 cm three times and averaged

*** Indirect calorimetry using a canopy hood will be performed for 30 minutes for measurement of basal energy expenditure, CO_2 production and O_2 consumption

ALT = Alanine Transaminase

AST = Aspartate Transaminase

SAMPLE COLLECTION

A total of 37ml (~8 teaspoons) of blood and 32ml (~7 teaspoons) of urine will be collected for the entire study. During visit 1 (Screening), 11ml (~2 teaspoons) of blood and 6ml (~1 teaspoon) of urine will be collected. During visit 2 (Dosing visit), 13ml (~3 teaspoons) of blood and 11ml (~3 teaspoons) of urine will be collected. During visit 3 (Post-treatment and final visit), 13ml (~3 teaspoons) of blood and 15ml (~3 teaspoons) of urine will be collected. If female subjects are actively menstruating at the time of study visit, urine tests will not be sent for biochemical or metabolomics analysis.

Blood and urine samples for laboratory testing at the clinical laboratory (Table 1) will be despatched for immediate analysis. Blood and urine samples for metabolomic profiling will be stored in the SGH CTRC at -80°C, and then sent to Duke-NUS laboratory for batch analysis.

Informed consent will be taken from participants regarding storage of biological materials. The blood and urine specimens will be coded and stored for future research if participants consent to this. The blood specimens will not be transferred out of Singapore.

Subjects who developed drug-related adverse reactions may require additional blood and urine taken for clinical assessment. The estimated amount required for these additional assessments are 10 ml (~2 teaspoons) of blood and 10 ml (~2 teaspoons) of urine.

LABORATORY MEASUREMENTS

A) Blood Chemistry

Screening and standard laboratory tests including full blood count, Potassium, Creatinine, ALT, AST, β-HCG (for female subjects of childbearing age), lipid panel, glucose and HbA1c will be measured using standard methods.

B) Metabolomic Profiling

Metabolomic profiling of lipids, acylcarnitines (ACs), organic acids and ketones will be measured using liquid and gas chromatography and tandem mass spectrometry (LC-MS/MS and GC-MS/MS) at the Duke-NUS metabolomics laboratory. Metabolomic analysis will be performed on serum (acyl-carnitines, amino acids and organic acids) as well as urine (organic acids).
ACs represent intermediaries of metabolic fuel oxidation and provides a "snap-shot" of *in vivo* metabolism at the cellular level. The measurement of the various long-, intermediate-chain ACs provide an indicator of fatty acid oxidation efficiency. Ceramides are toxic lipid intermediaries that are implicated in the pathogenesis of insulin resistance. Lactate, pyruvate, succinate, fumarate, malate, alpha-ketoglutarate and citrate are organic acids involved in TCA cycle activity. Beta hydroxybutyrate is a ketone which will also be measured with the organic acids.

C) Urine Protein Excretion

Urine ACR and PCR will be performed to quantify urine protein excretion.

4.3.3 VISIT 2: Initiation of Study Drug

Subjects who satisfy the inclusion and exclusion criteria will return to the CTRC within the next **14 days** for **visit 2** to receive the study drug, empagliflozin. Empagliflozin 10mg daily is to be taken orally for 30 ± 7 days. Subjects will take the first dose of empagliflozin under supervision of the clinical research coordinator at SGH CTRC.

Patients will be instructed to take empagliflozin once daily with water. To ensure a dose interval of about 24 hours, the medication should be taken at the same time every day, with or without food. If a dose is missed by more than 12 hours, that dose should be skipped and the next dose should be taken as scheduled. No double doses should be taken, and dose reductions are not permitted.

4.3.4 VISIT 3: 4-Week Post-Treatment and Final Visit

Visit 3 will take place **30 ± 7 days** from Visit 2. Subjects will be assessed to quantify changes in metabolic fuel utilization after treatment with empagliflozin. These changes are determined based on metabolomic profiling of acyl-carnitines, organic acids and amino acids and indirect calorimetry measurements. The list of procedures performed is listed in **Table 1**.

Subjects will take their final dose of study medication on the day of visit 3. Subjects will be asked to bring all trial medication containers (with or without any remaining tablets) with them to visit 3. Subjects who forget to return trial medication containers will be asked to bring them at a separate visit. The tablets will be counted by the PI, Co-I or study team members and compliance will be calculated according to the formula:

$$\text{Compliance (\%)} = \frac{\text{Number of tablets actually taken since visit 2}}{\text{Number of tablets which should have been taken in the same period}} \times 100\%$$

In addition, study subjects will be asked to fill up a Subject Treatment Diary to record the number of doses of empagliflozin 10mg taken. The Subject Treatment Diary will be reviewed by the PI, Co-I or study team member at visit 3.
The study drug will be discontinued upon completion of visit 3, and any remaining study drug tablets will be returned to the study team.

Should study subjects misplace any empagliflozin 10mg tablets between visit 2 and visit 3, the exact number of misplaced tablets will be resupplied to them by the study team.

Study drug will be discontinued for subjects upon occurrence of any adverse drug reaction to empagliflozin.

4.3.5 Post Study Follow up and Procedures

Subjects will continue their routine follow-up with their regular physicians upon completion of the final study visit. There will be no post study follow-up procedures.

4.4 Discontinuation/Withdrawal

4.4.1 Discontinuation Criteria

Study drug will be discontinued following completion of visit 3 (final study visit).

Study drug will be discontinued upon the development of any of the following adverse reactions:

1. Any allergic reaction to study drug
2. An increase in creatinine by > 50% compared to baseline
3. Ketoacidosis
4. Urosepsis and pyelonephritis
5. Fournier's gangrene

Individual patients will also be withdrawn from the trial if:

1. The patient withdraws consent, without the need to justify the decision.
2. If a patient becomes pregnant during the trial, the investigational drug will be stopped, the patient will be discontinued from treatment, and the patient will be followed up until birth or otherwise termination of the pregnancy.

All patients who drop out of the study after visit 1 (Consent & Screening), but before visit 2 (Dosing) will be considered a screening failure, and no further follow-up is required.

4.4.2 Discontinuation Visit and Procedures

Should any suspected study drug-related adverse effects occur, study subjects may contact the study coordinator. If required, they would be scheduled for a visit at SGH CTRC within the next 72 hours. They will be assessed by a member of the study team who is a registered medical practitioner for their suitability to continue with the study drug. Subjects may be referred back to their primary physician or other physicians as necessary upon the discovery of any drug-related adverse reaction to be given appropriate care under medical supervision until the symptoms of any adverse event resolve, or the subject's condition becomes stable. In the event of any medical emergency or urgency, subject will be referred to the Emergency Department.

Subjects who have taken at least one dose of the study drug and withdrew from this study (voluntary withdrawal or discontinuation due to adverse reaction), are encouraged to return for their third visit (30 ± 7 days from Visit 2) any and complete all planned study procedures as listed in **Table 1**. Subjects who had not taken any study drug will not be required to return for any subsequent study visits.

5 TRIAL MATERIALS

5.1 Trial Product (s)

Information on trial product (empagliflozin 10mg) is detailed in the prescribing information attached in Appendix 2.

5.2 Storage and Drug Accountability

Empagliflozin (Jardiance) will be stored in the original package in order to protect from light and moisture, at a room temperature not exceeding 30 °C. It will be stored securely in the investigator product cabinet at the SGH CTRC IP Room with restricted access. Study drug will be stored securely and the study coordinator will maintain a dispensing log. IP will be monitored by a 24-hour Temperature Monitoring System (TMS) and reports will be generated on a fortnightly basis. Alarms to any excursion will be triggered and the CTRC staff and FRS will be informed.

6 TREATMENT

6.1 Rationale for Selection of Dose

Dosage of empagliflozin is similar to that used for the treatment of type 2 diabetes mellitus in clinical practice. Eligible subjects will be given empagliflozin 10mg once daily to be taken orally for 30 ± 7 days.

6.2 Study Drug Formulations

Study drug will be dispensed as empagliflozin (Jardiance) 10mg film-coated tablets.

6.3 Study Drug Administration

Study drug is to be taken orally. Subjects will be warned to look out for the following symptoms and contact the study coordinator if they occur:

a) Development of allergic reactions (eg: new rash)
b) Symptoms suggestive of hypotension (eg: light-headedness)
c) Symptoms suggestive of ketoacidosis (eg: nausea, vomiting, abdominal pain, generalized malaise, shortness of breath)
d) Factors predisposing to ketoacidosis which may warrant discontinuation of empagliflozin (eg: acute febrile illness, reduced caloric intake due to illness or surgery)
e) Factors predisposing to acute kidney injury which may warrant discontinuation of empagliflozin (eg: reduced oral intake, fluid losses)
f) Symptoms suggestive of urinary tract infection (eg: fever, dysuria) and genital mycotic infection (eg: genital discharge or pruritus)
g) Symptoms suggestive of Fournier's gangrene (eg: fever, malaise, or tenderness, redness, or swelling of the genitals or the area from the genitals back to the rectum)

6.4 Specific Restrictions / Requirements

Individuals who are treated with insulin or in whom insulin treatment is clinically indicated will be excluded from this study.

6.5 Blinding

This is an open-label study.

6.6 Concomitant therapy

All medications (prescription and over the counter), vitamin and mineral supplements, and / or herbs taken by the participant will be documented.

7 SAFETY MEASUREMENTS

7.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

A serious adverse event (SAE) or reaction is any untoward medical occurrence that at any dose:

- results in or contributes to death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in or contributes to persistent or significant disability/incapacity or
- results in or contributes to a congenital anomaly/birth defect
- results in such other events as may be prescribed
- is a medical event that may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

7.2 Collecting, Recording and Reporting of Adverse Events and Serious Adverse Events to CIRB

Reporting of adverse events involves the PI submitting to the approving CIRB the completed SAE Reporting Form within the stipulated timeframe. PI is responsible for informing the institution representative (local SAE resulting in death), sponsor or regulatory bodies as required and appropriate.

Reporting timeline to CIRB:

- SAE that result in death, regardless of causality, should be reported immediately - within 24 hours of the PI becoming aware of the event.
- Local life-threatening (unexpected/ expected) SAE should be reported no later than 7 calendar days after the Investigator is aware of the event, followed by a complete report within 8 additional calendar days.
- Local unexpected SAE that are related events, but not life-threatening, should be reported no later than 15 calendar days after the investigator is aware of the event.
- An increase in the rate of occurrence of local expected SAE, which is judged to be clinically important, should be reported within 15 calendar days after the PI is aware of the event.
- Local expected SAE should be reported annually (together with Study Status Report for annual review).
- Local unexpected and unlikely related SAE that are not life-threatening should also be reported annually (together with Study Status Report for annual review).
- Local unexpected AE that are related events should be reported at least annually (together with Study Status Report for annual review).
- Non-local unexpected SAE that are fatal or life threatening and definitely/probably/possibly related should be reported not later than 30 calendar days after the PI is aware of the event.

7.3 Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

All SAEs that are unexpected and related to the study drug will be reported to HSA. All SAEs will be reported to HSA. Please refer to the HSA website for more information on Safety Reporting Requirements for Clinical Trials.

The investigator is responsible for informing HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. Follow-up information will be actively sought and submitted as it becomes available. For fatal or life-threatening cases, HSA will be notified as soon as possible but no later than 7 calendar days after first knowledge that a case qualifies, followed by a complete report within 8 additional calendar days.

7.4 Safety Monitoring Plan

Any adverse effects will be reported. The PI and Co-investigators will monitor and report to the CIRB and HSA of any SAE. There will be no DSMB. Risks of study drug will be continually reassessed throughout the study period. Closed monitoring meetings with the research team will take place every 4 weeks to discuss the data collected from monitoring reports.

7.5 Complaint Handling

Contact information of the study team and SingHealth CIRB will be provided to all study participants and available in the participant informed consent sheet.

The PI will be informed immediately about any complaint from study subjects. The PI will contact the relevant study subjects to resolve the complaint.

7.6 Incidental findings

Incidental findings are defined as findings which are discovered during the course of conducting the study, and which have potential health or reproductive importance to study participants, but are unrelated to the purposes, objectives or variables of the study. Participants will be asked to indicate whether they wish to be re-identified and notified in the event of an important incidental finding that is related to them.

Should participants agree to be re-identified and notified, the PI or other study team member delegated by the PI will explain the incidental finding to the participant, and advise them on the next appropriate step. The costs for any care needed to diagnose or treat an incidental finding will be borne by the study participant.

8 DATA ANALYSIS

8.1 Data Quality Assurance

Discrepancy and inconsistent data will be resolved by looking at the source document.

8.2 Data Entry and Storage

Data will be stored in paper form and electronically. Paper documents will be stored in a locked cabinet in the PI's office. All electronic data will be de-identified and password protected. Electronically entered data will be stored in the network drive of Singapore General Hospital. Source documents will be retained until at least 6-years after the end of the study, as per CIRB policy recommendation.

The PI, Co-Is and Research Coordinators will have access to the data for tracking and following up with the participants and for academic analysis purposes. For randomisation and statistical analysis purposes, data access may also be granted to Duke-NUS Biostatisticians.

9 SAMPLE SIZE AND STATISTICAL METHODS

9.1 Determination of Sample Size

The effect of short-term treatment with empagliflozin to cause a shift in metabolic fuel oxidation in DN is unknown. We anticipate that a significant shift in metabolic fuel utilization towards lipid oxidation will occur after 23 – 30 days of treatment with SGTL2i. 40 subjects with DN will be required. Assuming a screen failure rate of 1 in 3, a total of 60 subjects will need to be screened.

9.2 Statistical and Analytical Plans

For the measurement of metabolic fuel utilization and related parameters at baseline and post-treatment, values will be compared using the paired Student's *t*-test. If the data are not normally distributed, the appropriate transformation will be used or a non-parametric approach will be considered. Hypotheses testing will be non-directional (two sided), with values < 0.05 as significance. Software programs SPSS (version 21; SPSS, Chicago, IL) and GraphPad Prism (version 6, GraphPad Software) will be used for all statistical analysis. There will be no planned interim analyses.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator(s)/institution(s) will permit study-related monitoring, audits and/or IRB review and regulatory inspection(s), providing direct access to source data/document.

11 QUALITY CONTROL AND QUALITY ASSURANCE

All study team members will undergo protocol training to ensure adherence with the protocol and for accuracy in relation to source documents.

12 ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the Good Clinical Practice and the applicable regulatory requirements.

This final study protocol, including the final version of the Patient Information and Informed Consent Form, will be approved in writing by the Centralised Institutional Review Board (CIRB) and regulatory approval from Health Sciences Authority (HSA), prior to enrolment of any patient into the study.

The principle investigator is responsible for informing the CIRB and HSA of any amendments to the protocol or other study-related documents.

12.1 Informed Consent

Informed consent will be taken during Visit 1 at SGH CTRC. Consent will be taken by the the PI or investigators assigned by the PI. There will be a provision for a translator for non-English speaking subjects. Illiterate subjects have the option to have a family member present during the consent taking process. Vulnerable subjects will not be recruited for this study. In obtaining and documenting informed consent, the investigator will comply with the GCP guidelines and to the ethical principles that have their origin in the Declaration of Helsinki.

12.2 Confidentiality of Data and Patient Records

Confidentiality of study participants will be respected and all potential participants will be assigned a study ID. Data and samples collected from study participants will be de-identified.

13 PUBLICATIONS

Publication-related decisions will be in discussed among all investigators and collaborators but the final decision lies within the PI.

14 RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as CIRB records and other regulatory documentation will be retained by the P.I. in a secure storage facility. The storage will be in accordance with SingHealth CIRB guideline on Retention of Research Data and Records and will be stored for a minimum storage period of 7 years after the completion of the study. The records will be accessible for inspection and copying by authorized authorities.

15 FUNDING and INSURANCE

The study is funded by the Tanoto Initiative for Diabetes Research Award (Duke-NUS TIDR/2018/0010) for a 2-year period.

This study will be covered under the National Clinical Trial Insurance Policy

List of Attachments

Appendix 1 Blood sampling schedule

	TOTAL VOLUME	
Blood	**(ml)**	**(Teaspoon)**
Visit 1	11	2
Visit 2	13	3
Visit 3	13	3
TOTAL	37	8

Urine	**(ml)**	**(Teaspoon)**
Visit 1	6	1
Visit 2	11	3
Visit 3	15	3
TOTAL	32	7

Appendix 2 Jardiance (Empagliflozin) prescribing information

Appendix 3 References

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