Astronaut

Acute effects of Sodium-glucose coTRansporter-2 inhibition on renal OxygeNation and AUTonomic function in type 1 diabetes

Title

Astronaut - **A**cute effects of **S**odium-glucose co**TR**ansporter-2 inhibition on renal **O**xyge**N**ation and **AUT**onomic function in type 1 diabetes

Short title

Sodium-glucose cotransporter-2 inhibition and renal oxygenation in type 1 diabetes

Setting

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Summary

Background: Inhibiting the sodium-glucose cotransporter-2 (SGLT2) has been observed to reduce risk of cardiovascular events and kidney failure in type 2 diabetes. The exact mechanisms of the beneficial effects of SGLT2 inhibition (SGLT2i) are still unknown. Kidney hypoxia has been demonstrated in diabetic kidney disease and SGLT2i is thought to relieve hypoxia in the kidneys. Mitochondrial dysfunction and autonomic dysfunction might also contribute to kidney hypoxia.

Objective: The primary aim of the study is to assess the acute effects of SGLT2 inhibition on parameters reflecting oxygenation and oxygen consumption of the human kidney in persons with type 1 diabetes. Exploratory aims are to investigate acute changes in oxygen availability and oxygen access to the kidneys after SGLT2i. This include measures of peripheral blood oxygenation, mitochondrial function and autonomic function.

Methods: Acute intervention study with oral dapagliflozin given in two doses each of 50 mg or matching placebo as intervention. Kidney oxygenation and perfusion parameters will be assessed by blood-oxygen-dependant level magnetic resonance imaging. Mitochondrial function will be assessed by extracellular flux analysis on lymphocytes. Autonomic function will be assessed by measuring baroreflex sensitivity.

Design: Randomized, double blinded, placebo-controlled, cross-over intervention study.

Study population: Fifteen healthy controls are recruited by advertisement and 15 patients with type 1 diabetes recruited from Steno Diabetes Center Copenhagen.

Endpoints: Primary end-point: Renal cortical and medullary oxygenation (T_2*). Exploratory end-points: Renal cortical and medullary perfusion, renal artery flow, renal oxygen consumption, peripheral capillary oxygen saturation (SpO2), arterial oxygen partial pressure (PaO2), arterial oxygen saturation (SaO2), lymphocyte mitochondrial function, baroreflex sensitivity.

Timeframe: Inclusion of patients from January 2020. Last patient last visit January 2021. Data analysis completed spring 2021, presentation autumn 2021 and publications Winter 2021.

Background

Diabetic nephropathy is a lethal and common diabetic complication. Renal complications are associated with development of end stage renal disease (ESRD) and cardiovascular morbidity and mortality. Although the prognosis has improved with implementation of antihypertensive treatment and particularly with agents blocking the renin angiotensin aldosterone system (RAAS) (1), the prognosis is still poor. Thus, there is an urgent need for discovery of mechanisms behind the development and progression of nephropathy in terms of improving treatment and prevention of diabetic nephropathy.

Sodium-glucose cotransporter-2 inhibitors

The sodium-glucose cotransporter-2 (SGLT2) is selectively expressed in the human kidney, where it executes reabsorption of filtered glucose with a high capacity. As a therapeutic target, SGLT2 has been successfully engaged by orally active, selective agents. SGLT2 inhibition (SGLT2i) has a range of known physiological effects: Glycosuria, natriuresis, plasma volume reduction, blood pressure reduction, increased lipid oxidation and ketogenesis (2). However, only few studies have described renal physiologic effects of SGLT2 inhibition in humans, where it has been observed that hyperfiltration and renal plasma flow is reduced during clamped euglycemia in type 1 diabetes (3).

SGLT2i have been observed to reduce cardiovascular mortality and to be associated with slower progression of diabetic kidney disease in the EMPA-REG-trial (4, 5); to reduce risk of cardiovascular events and kidney failure in the CREDENCE-trial (6); to slow progression of albuminuria in the CANVAS-trial (7); and to reduce the risk of renal events in the DECLARE-trial (8). The SGLT2 inhibitor dapagliflozin, used in the DECLARE study, reach peak plasma concentration after two hours and based on glucose excretion, the renal effects seem to be present after a few hours. The half-life is approximately 17 hours (9). The exact mechanisms of the beneficial effects of SGLT2 inhibition in diabetes is still unknown, but several hypotheses have been suggested. Hypoxia might play an important role.

Renal hypoxia

The kidneys have a high oxygen consumption, needed for reabsorption of filtered solutes, mainly sodium. While the renal cortex has a high perfusion, the renal medulla has a low

perfusion and at the same time a high oxygen demand needed for sodium reabsorption in the ascending loop of Henle, making it more prone to hypoxia. The presence of hypoxia has been demonstrated in diabetic kidney disease compared to age-matched healthy controls (10). At the same time, there is strong evidence of tissue hypoxia playing a central pathogenic role in the development of chronic diabetic kidney disease (11, 12). This is supported by a recent study showing that renal cortical hypoxia predicts decline in renal function in subjects with chronic kidney disease (13).

In diabetes, a number of factors can act together and increase the risk of renal hypoxia (14):

1. hyperglycemia increases metabolism both by increasing the amount of glucose to be reabsorbed, but via hyperfiltration also the amount of sodium to be reabsorbed. The sodium-glucose cotransport is a very energy(oxygen)-consuming process.
2. sustained hyperglycemia increases oxygen demand by reducing the metabolic efficiency, due to mitochondrial dysfunction and reduced efficiency of electrolyte transport
3. increased angiotensin-II receptor activity and oxidative stress can further reduce the metabolic efficiency of electrolyte transport
4. Increased oxygen demands cannot be met by increased blood flow, because this will further increase metabolism by increasing the filtered load of solutes

<u>Blood-oxygen-dependant level magnetic resonance imaging</u>

Renal blood oxygenation can be measured with magnetic resonance imaging (MRI) by using the paramagnetic property of deoxyhemoglobin as a surrogate measure. The blood oxygen level magnetic (BOLD) imaging signal is expressed by the transverse relaxation time (T_2*). T_2* measurements do not differentiate between oxygen delivery and oxygen consumption and for this reason it has been suggested that measurement of renal blood flow is mandatory to interpret renal T_2* values. Renal artery flow can be measured using phase contrast (PC) MRI. Renal tissue perfusion can be measured noninvasively with MRI using arterial spin labelling (ASL) (15). Renal oxygen consumption has not previously been measured but hypothetically

it can be estimated with Q-flow combined with measurement of BOLD in the renal artery and vein.

Mitochondrial function

Mitochondria play an important role in aerobic metabolism. Thus, it is assumed that generalized or local mitochondrial dysfunction can contribute to an increased oxygen demand in the kidneys. Mitochondrial dysfunction can be quantified through extracellular flux analysis on peripheral blood mononuclear cells (PBMCs) which are easily accessible from a blood sample. This method gives information about a variety of measures of mitochondrial function including basal respiration, ATP production, proton leak and maximal respiration. Technical details and interpretive value of this method have been well described (16).

Autonomic function

Cardiovascular autonomic dysfunction is frequent in diabetes and a risk factor for mortality in persons with type 1 diabetes and nephropathy (17). A relevant question is whether some of the positive effects of SGLT2i on the cardiovascular- and renal system are mediated by interaction with the autonomic nervous system in the kidney or directly in the central nervous system. Several findings indicate cross-talk between the sympathetic nervous system and SGLT2 regulation (18):

1) The target of SGLT2 inhibitors is the same as with sympathetic nerves, i.e. renal tubular epithelial cells, where efferent sympathetic fibres promote tubular sodium reabsorption
2) Increased SGLT2 expression have been induced by the sympathetic neurotransmitter noradrenaline in human renal proximal tubule cells

Autonomic function can be quantified by measuring baroreflex sensitivity, which can be determined by spontaneous fluctuations in the RR interval and systolic blood pressure (19).

What this study adds

With this study we will gain important knowledge aimed for the prevention and treatment of diabetic kidney disease in patients with type 1 diabetes:

1) The identification of mechanisms related to the beneficial effects of SLGT2 inhibition may provide new horizons for other therapeutic and preventive targets;

2) The assessment of the relation between kidney hypoxia, mitochondrial function and autonomic function may contribute to a better understanding of the pathophysiology of diabetic kidney disease in type 1 diabetes, again possibly leading to new therapeutic and preventive strategies.

This will benefit all patients with type 1 diabetes as well as providing the opportunity for new treatment options. The economic burden of diabetic nephropathy is huge and thus, strategies preventing progression of the disease could have a significant economic impact on society.

Specific aims

The primary aim of the study is to assess the acute effect of SGLT2 inhibition on parameters reflecting oxygenation and oxygen consumption of the human kidney in persons with type 1 diabetes. The exploratory aims are to investigate changes in oxygen availability and oxygen access to the kidneys after SGLT2i. This includes measures of peripheral blood oxygenation, PBMC mitochondrial function, autonomic function and inflammatory markers.

Hypothesis

We hypothesize that SGLT2 inhibition acutely reduces the kidneys demand for oxygen by reducing renal metabolism due to blocked sodium-glucose transportation, and hyperfiltration. We hypothesize that the following changes can be demonstrated after institution of SGLT2 inhibition:

1) Lowering of renal artery flow (PC-MRI)
2) Lowering of renal cortical perfusion and the ratio of cortical/medullary perfusion (ASL-MRI)
3) Better medullary oxygenation: Lowering of the renal medulla T_2* (BOLD-MRI)
4) Decrease of renal oxygen consumption (Q-flow combined with measurement of BOLD in renal artery and vein)

We further hypothesize that autonomic function will improve, and that peripheral blood oxygenation and mitochondrial function will both increase after institution of SGLT2 inhibition.

Study population

Fifteen healthy controls recruited through an advertisement in the local newspapers and 15 persons with type 1 diabetes recruited from Steno Diabetes Center Copenhagen according to the study in-and exclusion criteria.

Inclusion criteria healthy controls:

- Written informed consent must be provided before participation
- Male or female patients > 18 years of age
- Capable of lying in a MR-scanner for two hours

Inclusion criteria persons with type 1 diabetes:

- Written informed consent must be provided before participation
- Male or female patients >18 years of age with a diagnosis of type 1 diabetes (WHO criteria)
- Urinary albumin creatinine ratio (UACR) ≥30 mg/g in 2 out of 3 consecutive samples (albuminuria) prior to randomization assessed from electronic laboratory database.
- Capable of lying in a MR-scanner for two hours

Exclusion criteria for all:

- Non-diabetic kidney disease as indicated by medical history and/or laboratory findings
- Renal failure (eGFR<15 ml/min/1.73m2), dialysis or kidney transplantation
- Treatment with beta-blocking medication
- Uncontrolled arrhythmia, 2. or 3. degree AV-block or sick sinus syndrome - assessed from a standard 12-lead electrocardiogram
- Pregnancy or breastfeeding (urine HCG is performed on all fertile women)
- Systolic blood pressure < 90 or > 200 mmHg
- Patients who, in the judgement of the investigator, is incapable of participating
- Exclusion criteria for MRI
    - Claustrophobia
    - Known heart disease

  - Known lung disease
  - Have had surgery the past six weeks
  - Have foreign bodies of metal in the body (e.g. pacemaker, metal plates, metal screws)
- Exclusion criteria for arterial blood gas sampling (only patients with type 1 diabetes)
  - Absent pulse
  - Raynauds syndrome
  - Buergers Disease (thromboangiitis obliterans)
  - Inadequate or interrupted circulation
  - Anticoagulation treatment
  - Coagulopathies (hypo or hyper coagulable states)
  - Arterial atherosclerosis
  - Insufficient collateral perfusion
  - Partial or full thickness burns over the cannulation site
  - Synthetic arterial or vascular grafts or infection at the proposed site of cannulation

Patients with type 1 diabetes will have the possibility to participate in the study without getting arterial blood gas sampling.

- Healthy control exclusion criteria
  - The subject has a clinically significant abnormal laboratory value and/or clinically significant medical or psychiatric illness.
  - The subject has evidence of clinically significant gastrointestinal, cardiovascular, hepatic, renal, hematological, neoplastic, endocrine, neurological, immunodeficiency, pulmonary, or other disorder or disease.

<u>Patient withdrawal</u>

In case of pregnancy (or desire for pregnancy), female subjects are withdrawn.

Lack of compliance to any of the important study procedures in the discretion of the investigator.

Onset of any disorder considered to compromise the safety by participating in the study.

Unacceptable adverse effects in the discretion of the investigator.

Withdrawal on participants request will be accepted at any time without further justification or consequence to regular treatment at the Steno Diabetes Center Copenhagen.

Withdrawn patients will be replaced by patients recruited in the same manner as described in this protocol.

Recruitment

Information about civil registration number, phone number, address, diagnosis of T1D and historic levels of urine albumin on outpatients at Steno Diabetes Center Copenhagen is provided by the treatment-responsible doctor and passed on to the study investigator. This information is used for identifying possible participants and recruitment. First contact will be a letter containing the written participant information and the brochure: "Your rights as a participant in biomedical research". In the letter, possible participants will be invited to the information meeting at Steno Diabetes Center Copenhagen. The letter contains telephone number and email for the project-responsible doctor. Non-responders will be followed up by a telephone contact approximately two weeks later. All who wish to participate in the study are invited for an information visit.

Healthy controls will be recruited by advertisement in the local newspapers. In the advertisement, there will be phone number and email of the investigators for contact.

Endpoints

Primary end-point

- Renal oxygenation (T_2*)

Exploratory end-points

- Renal cortical and medullary perfusion
- Renal artery flow
- Renal oxygen consumption
- Peripheral capillary oxygen saturation (SpO2)
- Arterial blood gasses
    - Arterial blood oxygen partial pressure (PaO2)

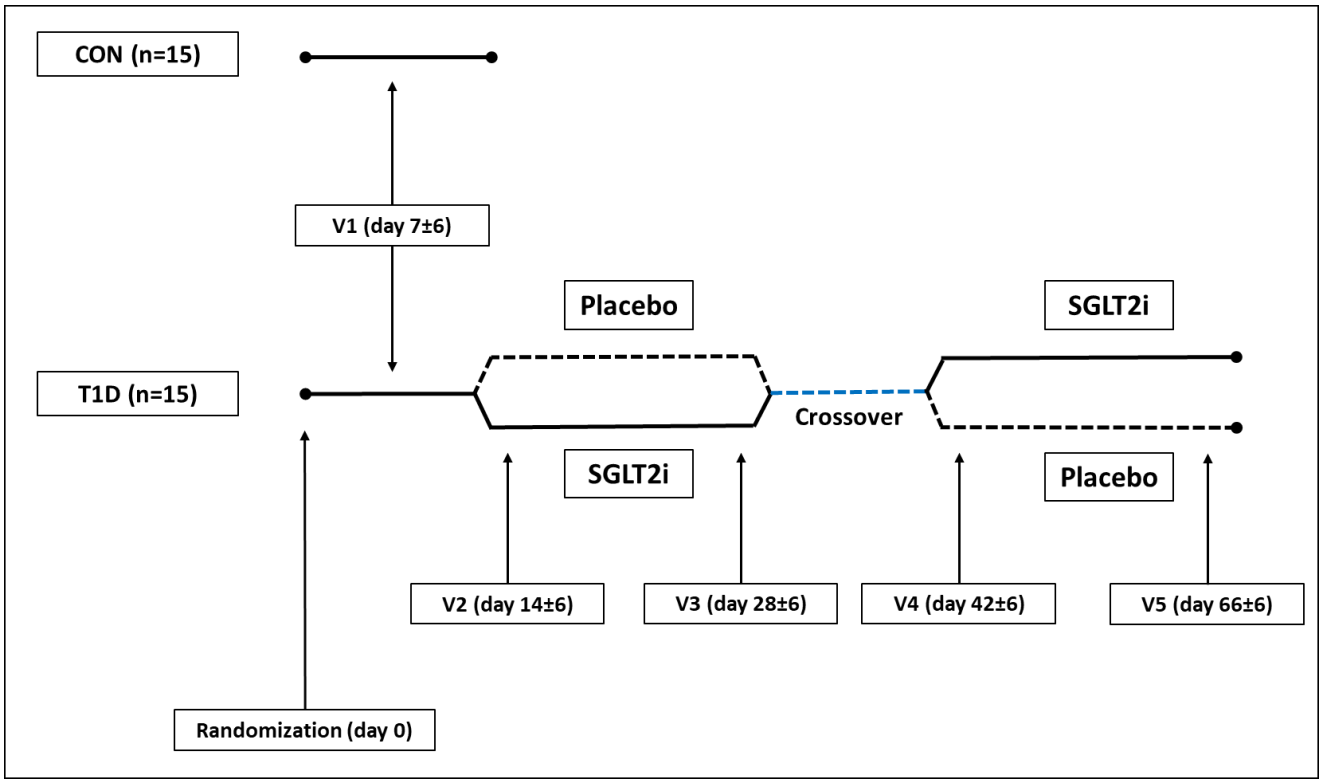
- Arterial blood oxygen saturation (SaO2)

- PBMC mitochondrial function
- Circulating inflammatory markers
- Baroreflex sensitivity

Study design

Randomized, double blinded, placebo-controlled, cross-over intervention study in patients with type 1 diabetes. Baseline results will be compared to healthy controls.

Figure 1



Flowchart of study design. Healthy controls (CON) will only come for baseline measurements on V1 and V2, except blood gas analysis, which will only be done in randomized patients.
Patients with type 1 diabetes (T1D) will be randomized to SGLT2i or placebo for Visits 1+2 and crossed over for Visits 3+4.

Comparative treatment regimes

Patients in the active arm will be treated with dapagliflozin 50 mg once on site for V2 and once at home on the evening before V3.

Randomization and blinding

Patients will be randomly assigned in a 1:1 ratio to one of the following groups:

- Dapagliflozin for V2 and V3 and placebo for V4 and V5
- Placebo for V2 and V3 and dapagliflozin for V4 and V5

Group allocation is concealed to patients as well as investigators. 60 sequentially numbered, opaque, sealed envelopes will be produced by Glostrup Apotek. All persons involved in the conduct of the study are blinded to the randomization code. Randomization codes and envelopes are stored securely at the study site available only for the unblinded site staff in charge of randomizing subjects and dispensing study products to subjects. Sealed codes are marked according to randomization code and distributed according to a pre-distributed order. Should unblinding of a study participant be necessary because of an emergency, a dedicated person at Steno Diabetes Center Copenhagen, not involved in the study, will perform the procedure. Alternatively, the Principal investigator will be able to perform unblinding.

Description of investigational drug and placebo drug

Investigational drug:

Forxiga®, dapagliflozin 10 mg film-coated tablet.

For further information please refer to:

https://www.ema.europa.eu/en/documents/product-information/forxiga-epar-product-information_en.pdf.

Common side effects include hypoglycemia, hypotension, elevated hematocrite, dyslipidemia, back pain, dizziness, skin rash, urinary tract infection, vulvovaginitis and dehydration. Very rare incidents of ketoacidosis have been observed. Side effects have only been observed after use in longer periods and not in single-dose usage, as planned in the present study. A dose of 50 mg has been chosen to achieve optimal efficacy. Once-per-day doses of dapagliflozin for 12 weeks of 2.5 mg, 5 mg, 10 mg, 20 mg and 50 mg have been

demonstrated to be relatively safe across the mentioned doses (20) and no apparent risk is expected from instituting two single-doses of 50 mg dapagliflozin.

Placebo drug:

The composition equals the composition of Forxiga® – just with the active ingredient omitted. Active drug and placebo are similar in appearance and smell.

Drug storage

Forxiga® does not require any special storage conditions

Drug accountability

Patients will be instructed to return all wholly or partially used drugs and study medicine packaging. Drug accountability will be performed at the investigation site.

Study duration

Eight weeks for randomized patients. Two weeks for healthy controls.

Methods

Blood-oxygen-dependent level magnetic resonance imaging

Scanning will be performed on a 3-T Philips Achieva scanner using the scanners body transmit and a four-element SENSE cardiac receive coil. Subjects will be scanned using MRI sequences to map ASL perfusion and BOLD T_2* in the kidneys and to measure flood flow in the renal artery. Renal oxygen consumption will be measured using Q-flow combined with measurement of BOLD in the renal artery and vein. All measurements will be performed twice.

Mitochondrial function

Mitochondrial function will be quantified by using the Seahorse 96XF Analyzer (Agilent Technologies, Santa Clara, CA, USA) that can measure oxygen consumption rate and extracellular flux in real time in a 96-well plate in intact peripheral blood monocytes (PBMCs). Extracellular flux is changes in oxygen and proton concentrations in the media surrounding the PBMCs. From this, the relative state of aerobic and glycolytic metabolism can be

determined. Thus, the Seahorse assays can quantify enzyme defects, including oxidative phosphorylation, glycolytic pathway abnormalities and fatty acid oxidation pathway abnormalities (21).

Circulating inflammatory markers

Inflammatory markers will be measured on venous blood with a validated assay including 92 inflammation-related human protein biomarkers simultaneously (Olink, Uppsala, Sweden) (22).

Baroreflex sensivity

Baroreflex sensitivity is defined as the change in heart-rate in relation to change in blood pressure. It is considered a sensitive measurement of cardiovascular autonomic function (19). Baroreflex sensitivity will be determined from spontaneous fluctuations in the RR interval and systolic blood pressure. We will use a method earlier described in detail (23, 24).

Blood samples

From healthy controls, a total of 21 ml of blood will be taken at V1. From randomized patients, a total of 21 ml of blood will be taken at V1, 3 ml blood at V2, 11 ml blood at V3, 3 ml blood at V4 and 11 ml blood at V5. The material will only be used for analysis in the Astronaut study and will be destroyed at the end of the study.

Clinical chemistry will include measurement of p-HbA1c, p-potassium, p-sodium, p-creatinine, p-albumin, haemoglobin, leucocytes, thrombocytes, haematocrit, p-ALT, p-INR (international normalized ratio), p-lipids (total-, HDL-, and LDL cholesterol and triglycerides). Kidney function (eGFR) will be calculated using the CKD-EPI equation from p-creatinine. Blood samples for clinical chemistry will be analysed on the day of collection and destroyed immediately after.

Peripheral blood monocytes will be purified from blood to examine mitochondrial function at Steno Diabetes Center Copenhagen on V1, V3 and V5. Samples will be analysed on the day of collection and destroyed immediately after.

A research biobank of 4 ml blood per participant will be used for analysis of inflammatory markers. A research biobank means that the samples are frozen, analysed by the end of the study (February 1st 2022) and hereafter destroyed.

Blood gas analysis include arterial blood oxygen partial pressure (PaO2) and arterial blood oxygen saturation (SaO2). Blood gas analysis will be performed on site at Rigshospitalet Glostrup on V2 and V4. Samples will be analysed on the day of collection and destroyed immediately after. Blood gas analysis will only be performed on randomized patients.

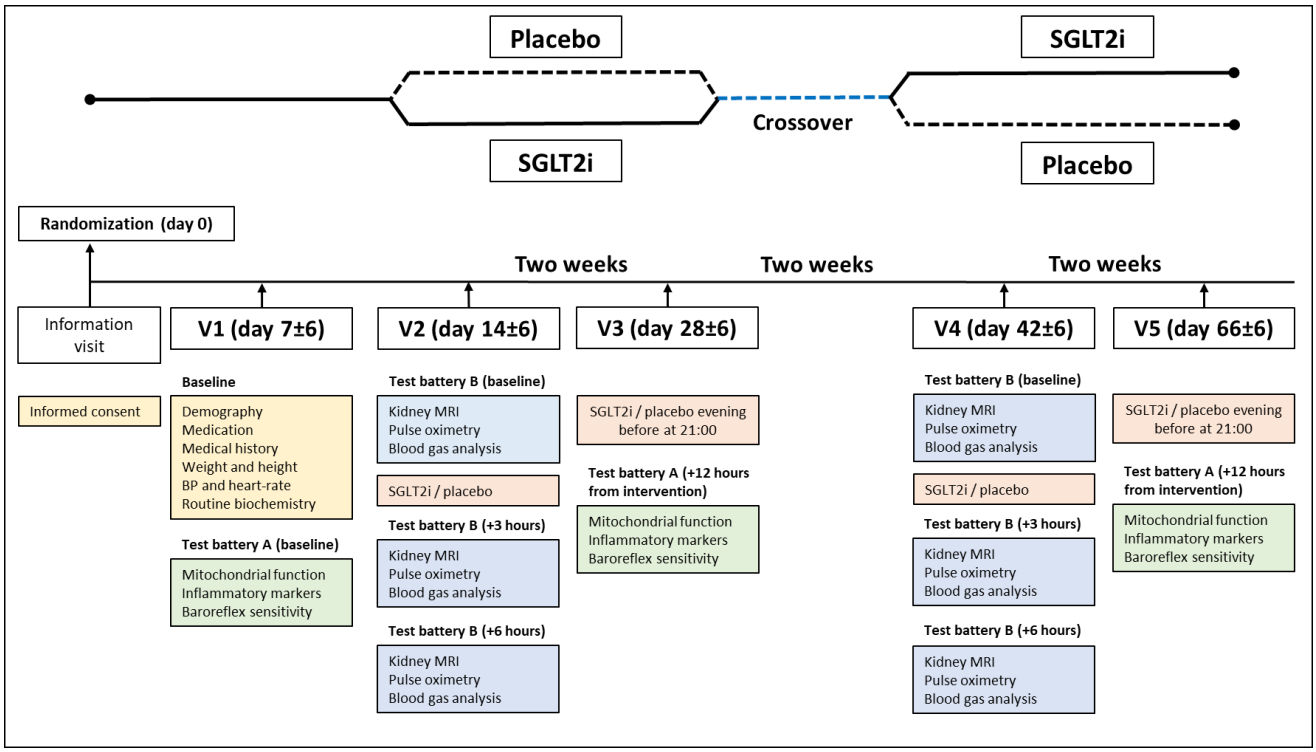
Biobank for unspecified future research

Study participants will be asked if they are willing to donate blood and urine to a biobank for unspecified future research. This biological material will only be used for future research after specific approval from the regional ethical committees.

Study visits

Please note that healthy controls will only participate for the information meeting, V1 and the baseline measurements of V2, except from blood gas analysis. Figure 2 shows an overview of study visits.

Figure 2



Overview of study visits. Patients with type 1 diabetes come for information visit and V1-V5.

Healthy controls come for information visit and baseline measurements of V1 and V2, except blood gas analysis.
BP = blood pressure, MRI = Magnetic resonance imaging, SGLT2i = Sodium-glucose cotransporter-2 inhibitor.

Information meeting (day 0) at Steno Diabetes Center Copenhagen

Information about the study background, design and aspects of the study will be given by the investigator. After consideration, the participants interested in participating in the study are asked to sign an informed consent form. After signed informed consent, patients will be randomized. See section "Participant information/informed consent" for details on informed consent.

Visit 1 (day 7±6) at Steno Diabetes Center Copenhagen

The study participant will come fasting from the morning and abstinent from medication, alcohol and tobacco use except long-acting insulin, 24 hours before the visit. The study participant is not allowed to do strenuous exercise 24 hours prior to the examination. Visit 1 will take approximately two hours. The following questions, measures and samples are obtained:

- Demographic information, medication and medical history are obtained from electronic medical journal and by interview with the study participant
- Weight and height for calculation of body mass index (BMI)
- Office blood pressure and heart rate
- Routine biochemistry
- Test battery A (baseline)
    - Analysis of mitochondrial function of peripheral blood monocytes
    - Sample kept in research biobank for analysis of inflammatory markers
    - Baroreflex sensitivity assessed by continuous ECG and blood pressure

Visit 2 (day 14±6) at Rigshospitalet Glostrup

The study participant will come fasting from the morning and abstinent from medication, alcohol and tobacco use except long-acting insulin, 24 hours before the visit. The study

participant is not allowed to do strenuous exercise 24 hours prior to the examination. Visit 2 will take approximately eight hours. The following measures will be obtained:

- Test battery B (baseline)
    - MRI session with two measurements of: Renal perfusion, renal artery flow, renal oxygen consumption and T_2* respectively
    - Peripheral blood oxygen saturation by pulse oximetry
    - Blood gas analysis

A standardized morning meal will be given together with the intervention: Institution of 50 mg SGLT2i/placebo. Exactly three hours after intervention, test battery B will be repeated:

- Test battery B (+3 hours)
    - MRI session with two measurements of: Renal perfusion, renal artery flow, renal oxygen consumption and T_2* respectively
    - Peripheral blood oxygen saturation by pulse oximetry
    - Blood gas analysis

Then a standardized lunch will be given. Exactly six hours after intervention, test battery B will be repeated:

- Test battery B (+6 hours)
    - MRI session with two measurements of: Renal perfusion, renal artery flow, renal oxygen consumption and T_2* respectively
    - Peripheral blood oxygen saturation by pulse oximetry
    - Blood gas analysis

Finally, an optional dinner will be offered. See table 1 for a detailed timetable over visit 2.

Table 1

Time	Patients with type 1 diabetes	Healthy Controls
08:00	Kidney MRI (baseline)	Kidney MRI (baseline)
08:30	Blood oxygen saturation (baseline)	Blood oxygen saturation (baseline)
08:40	Arterial blood sample and analysis (baseline)	-
09:00	Intervention: 50 mg SGLT2i/placebo	-
09:00	Standardized morning meal	Standardized morning meal
12:00	Kidney MRI (+3 hours from intervention)	-
12:30	Blood oxygen saturation (+3 hours)	-
12:40	Arterial blood sample and analysis (+3 hours)	-
13:00	Standardized lunch	-
15:00	Kidney MRI (+6 hours from intervention)	-
15:30	Blood oxygen saturation (+6 hours)	-
15:40	Arterial blood sample and analysis (+6 hours)	-
16:00	Optional dinner	-

Timetable over visit 2

Visit 3 (day 28±6) at Steno Diabetes Center Copenhagen

The study participant will be instructed to take 50 mg dapagliflozin / placebo at the time 21:00 on the evening before visit 3. The study participant will come fasting from the morning and abstinent from medication, alcohol and tobacco use except long-acting insulin, 24 hours before the visit. The study participant is not allowed to do strenuous exercise 24 hours prior to the examination. Visit 3 will take approximately one hour. The following measures and samples are obtained:

- Test battery A (baseline)
    - Analysis of mitochondrial function of peripheral blood monocytes
    - Sample kept in research biobank for analysis of inflammatory markers
    - Baroreflex sensitivity assessed by continuous ECG and blood pressure

Visits 4 (day 42±6) and 5 (day 66±6)

Apart from study medication/placebo, study visits 4 and 5 will be identical to visits 2 and 3 but crossed over in terms of SGLT2i/placebo. Table 2 shows study timetable for randomized patients.

Table 2

Visit	Information visit	V1	V2	V3	V4	V5
Site	SDCC	SDCC	RHG	SDCC	RHG	SDCC
Week	0	1	2	4	6	8
Day	0	7	14	28	42	66
Tolerance (± days)		±6	±6	±6	±6	±6
General						
Informed consent	x					
Assessment of in- and exclusion criteria	x					
If patient: Randomization	x					
Demography		x				
Concomitant medication		x				
Medical history		x				
Weight and height		x				
BP and heart-rate		x				
Routine biochemistry		x				
Endpoints						
Mitochondrial function		x		x		x
Inflammatory markers		x		x		x
Baroreflex sensitivity		x		x		x
Kidney MRI			x		x	
Pulse oximetry			x		x	
Blood gas analysis			x		x	
Safety						
Adverse events			x	x	x	x
Study medication						
Drug accountability				x		x

Study timetable for randomized patients. SDCC = Steno Diabetes Center Copenhagen, RHG = Rigshospitalet Glostrup.

Assessment of efficacy

In order to assess the acute physiological response of inhibiting SGLT2 in the proximal tubule, kidney MRI parameters, pulse oximetry and blood gas analysis are obtained before intervention (baseline), three hours after intervention and six hours after intervention. In order to assess the subacute, systemic physiological response, PBMC mitochondrial function, inflammatory markers and baroreflex sensitivity are obtained before intervention (baseline) and 12 hours after intervention. Baseline levels will be compared to those of healthy controls.

Response to dapagliflozin in outcomes in randomized patients will be compared to response to placebo.

Assessment of safety

Definition of SAE, SAR and SUSAR

Serious Adverse Event (SAE):

A Serious Adverse event is an experience that treatment results in any of the following:

- Results in death
- Is life threatening (note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe)
- Requires inpatient hospitalization or results in prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect, or
- Is an important medical event that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgement, they may jeopardise the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Suspicion of transmission of infectious agents must always be considered an SAE.

Serious Adverse Reaction (SAR):

A SAE for which a causal relationship to the study medication is at least possible i.e. a causal relationship is conceivable and cannot be dismissed.

Suspected Unexpected Serious Adverse Reaction (SUSAR):

A SAE which is unexpected and regarded as possibly or probably related to the study medication by the Investigator.

Reporting of SAE SAR and SUSAR

SAEs, SARs, SUSARs will initially be reported to the sponsor within 24 hours. Previous non-serious AEs, which becomes SAEs follows the reporting of SAEs.

Sponsor must ensure that all information on SUSARs that are fatal or life-threatening is recorded and reported to the Danish Medicines Agency (on their web-based forms) as soon as possible and no later than 7 days after the sponsor became aware of such possible side effects. Within 8 days after reporting the sponsor must notify the Danish Medicines Agency all relevant information about the sponsor and investigator's response to the alert and consequence for the study conduct.

All other SUSARs will be reported to the Danish Medicines Agency within 15 calendar days after the sponsor became aware of them. Furthermore, the investigator should notify AstraZeneca when expediting SARs or SUSARs to health authorities and should report all SARs related to the product to the local AstraZeneca affiliate safety department. The submission to AstraZeneca must be within day 15 from the investigator’s first knowledge about a valid case. Where required in national law the investigator should also expedite SARs or SUSARs, to Independent Ethics Committee (IEC)/Institutional Review Board (IRB).

All SUSARs will be unblinded before reporting to the authorities. All investigators have the authority to immediately unblind study medication if necessary.

Data on other AEs, including abnormal laboratory values as assessed by the investigator as clinically significant will be collected and recorded on standardized AE forms at each contact. These data are reported to relevant authorities in accordance with applicable laws and ICH-GCP guidelines.

When reporting SAEs and SARs the following parameters must be recorded: study name, patient identification (subject number, initials, sex, age), event (preferably a diagnosis), investigational medicinal product (IMP), reporter identification (name or initials), causality, outcome

Definition of adverse event

Any untoward medical occurrence in a patient or clinical investigation subject administered/using a product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not considered related to the product.

Reporting of adverse event

All events meeting the definition of an AE must be collected and reported. This includes events from the first study-related activity after the subject has signed the informed consent until the end of the study. When reporting Adverse Events the following parameters must be recorded: study name, patient identification (subject number, initials, sex, age), event (preferably a diagnosis), IMP, reporter identification (name or initials), causality, outcome. Timelines for reporting of AE´s is described in study timetable.

Assessment of adverse event

The following three definitions are used when assessing the severity of an AE: Mild (no or transient symptoms, no interference with the subject´s daily activities), Moderate (marked symptoms, moderate interference with the subject´s daily activities) and Severe (considerable interference with the subject´s daily activities; unacceptable).

The following terms are used when assessing the causality between an AE and the IMP: Probable (good reason and sufficient documentation to assume a causal relationship), Possible (a causal relationship is conceivable and cannot be dismissed) and Unlikely (the event is most likely to ethology other than the IMP (Forxiga®).

Reporting at the end of the study

A final report on the AEs and SAEs is sent to the Danish Medicines Agency, the Regional Scientific Ethics Committee and to AstraZeneca within 12 months after the last patient has completed the project.

Statistical considerations

Sample size

The primary goal is to test the hypothesis that SLGT2 inhibition will improve renal oxygenation with a lowering of medullary T_2*, as a marker of increased oxygenation. A power calculation was performed, based on data available from the study by Haddock. et al. (15). The mean ± SD resting medullary T_2* was 23 ± 4. Assuming that SGLT2 inhibition will improve oxygenation 20%, the sample size required to demonstrate a significant effect with a power of 80% and a type 1 error of 5%, is 13 subjects per group. To account for technical difficulties, we included 15 in each group. Power calculation was performed using the power statement implemented in the SAS enterprise software 7.1 (SAS Institute, Cary, NC, USA).

Analysis

All characteristics will be presented as means with standard deviation (SD) or if skewed distributions, as medians with interquartile range (IQR). For baseline characteristics, chi-squared test and Fischer's exact test will be used when appropriate. For testing group differences, unpaired student's t-test will be used. For testing the effect of the intervention, linear regressions analyses will be performed adjusting for baseline values of outcome variables. To fulfil the requirement of a normal distribution of the model residuals, determinants will be log-transformed where appropriate. Statistical significance is inferred as a two-tailed P-value < 0.05. All analyses will be performed in SAS enterprise 7.1.

Data management

Source data identification and source data verification

All clinical study information in this study will be recorded, handled, and stored in a way that allows it accurate reporting, interpretation and verification.

Sponsor/investigator will provide direct access to source data/documents for study related monitoring, audit, IEC/IRB review, and regulatory inspection. Sponsor has made an agreement with the GCP-unit at the University of Copenhagen, Denmark, who will be monitoring the study in accordance with the ICH guidelines for GCP.

Subject data protection

There will be a transfer of information from the doctor responsible for the person in the clinic to the doctor responsible for the research of relevance to study participation and safety in the personal record. All information on study subjects is protected according to law on processing of personal data and the law of health. None of the study related blood samples or data will be stored or analyzed in countries outside the EU.

Data handling

Before initiating the study acceptance from the Danish Data Protection Agency will be obtained. A separate case report form (CRF) will be prepared for each study participant. All health-related matters and sensitive personal data will be handled in accordance with the Danish "act on Processing of Personal Data".

All health-related matters and sensitive personal data (CRF, blood test result etc.) will be depersonalized. All participants will be given a study number referring to their personal information, which will be stored securely and separately. Adequate blinding of all personal data during data processing and publication will be ensured.

Data will be stored in coded form in 15 years after last patient last visit according to recommendations from the Data Protection Agency after which it will be transmitted to the Danish Data Archives.

Administrative procedures

Insurance

All participants are covered by the public patient insurance at Steno Diabetes Center Copenhagen (Patientforsikringen).

Ethics committee

The trial will be carried out in accordance with the Helsinki Declaration, EU Directive on good clinical practice (GCP) and ICH-GCP guidelines after approval by the Regional Scientific Ethical Committee, Danish Medicines Agency and the Data Monitoring Board. The study will be registered on www.clinicaltrials.gov and monitored by the GCP-unit at the University of Copenhagen, Denmark. Permission for third person to have access to patient data will be

obtained at information visit. This will provide access to source data and relevant documents in connection with monitoring, inspections and audits to relevant authorities.

Regulatory affairs

Study approval will be obtained from national authorities (The Ethics Committee at Region Hovedstaden, Denmark, the Danish Medicines Agency and the Danish Data protection Board) before commencement of the Study, as applicable according to local regulations. Notifications and reports will be filed according to ICH E6(R1): GCP: Consolidated guideline, CPMP/ICH/135/95, and national guidelines including Clinical Trials Directive 2001/20/EC.

Study monitoring

The Study will be monitored by the GCP-unit at the University of Copenhagen, Denmark.

Study audits and inspections

The patients will be informed in writing about the possibility of audits and/or inspections. Audit and/or inspections might be performed by the hospital institutional review board or regulatory authorities. In these cases, patient records will be required and reviewed.

Financing:

The study is a sub-study of the PROTON project, which is sponsored by a grant of 60 mio. DKK from the Novo Nordisk Foundation Grant number: NNF14OC0013659 and is expected to cover all costs related to the project. The budget assigned for the study covers 1.265.653 DKK. The grant-money is held on a research account administered by Steno Diabetes Center Copenhagen. No financial compensation will be given for patients participating in the study. Healthy controls will be offered 500 DKK (taxable) for participation in the study. Documented transportation expenses for all participants will be covered after the lowest applicable rate in Region Hovedstaden. The investigators have no financial conflict of interest in relation to the procedures or investigations in the study.

Ethical considerations:

Dapagliflozin is approved for the treatment of type 1 diabetes and type 2 diabetes and clinical trials have shown beneficial effects of dapagliflozin treatment on glycaemic control, weight and blood pressure. In addition, cardiovascular outcome trials have shown long-term cardioprotective and renoprotective effects. As this is a single-dose study, these benefits are not expected to occur in the participants.

Study participants will have the benefit of the individual results from kidney scans, blood tests and the autonomic function assessment, which are not necessarily carried out routinely at Steno Diabetes Center Copenhagen. Individual results will be given by the project responsible doctor. The participants' right not to know their own data will be respected.

Regardless of the outcome, the results and knowledge obtained from the project is believed to contribute to a better understanding of the mechanisms behind development and progression of diabetic nephropathy and thereby, in general, contribute to a possible improved treatment of diabetic kidney disease in the long term. This will benefit all patients with diabetes worldwide. In the opinion of the investigators it can be concluded that potential discomforts and risks are compensated by the expectable advantages of conducting this study.

The study may be stopped by the investigator if new information appears about the medicine that causes safety problems. Subjects will be informed accordingly.

Patient risks and management of complications

The risk is considered very small with treatment with two single doses of 50 mg, even if these doses are larger than usually applied daily doses (10 mg per day), as side effects have not been observed with this or higher doses (up to 500 mg as single dose). After longer periods of using dapagliflozin, the following side-effects have been observed: Hypoglycemia, hypotension, elevated hematocrite, dyslipidemia, back pain, dizziness, skin rash, urinary tract infection, vulvovaginitis and dehydration. Very rare incidents of ketoacidosis have been observed. If the mentioned side-effects against all expectations should occur, it is common for them, that they are transient or simple to manage with standard treatment methods. Blood sampling by arterial puncture and venous puncture can cause a short pain, a small risk of hematoma and a minimal infection risk at the puncture site. Arterial puncture is also

associated with a minimal risk of arteriospasm, nerve damage, fainting and thrombosis (25). The most feared complication is irreversible ischemic damage distally for the thrombosis-site. This will only happen if revascularization is not achieved before six hours after the vascular occlusion. Due to access to nearby vascular surgery, irreversible ischemic damage is not considered a realistic ultimate consequence of potentially occurring thromboses during the present study. Usually there is no discomfort associated with MRI scans. Short-term transient side-effects can be experienced: Slight increase in body temperature (< 0.6 °C), touch sensations, dizziness, nausea and taste of metal. Besides this, it can feel claustrophobic to lie in the scanner.

Complications that might occur will be managed and followed up relevantly by the investigators.

Participant information/informed consent

The participants are informed that participation in this research project is entirely voluntary. For all participants, the oral information is given at the information meeting by the investigator in calm surroundings. These surroundings are secured by having the meeting in a suited room with the possibility of closing the door. The participant is entitled to ask questions and bring a friend or relative to the information meeting, as described in the written information. Before informed consent is signed, the participant will be given the opportunity for at least 24 hours for consideration. In case the participant, after having been asked, does not express a need for further time for reflection, the informed consent is obtained at the information meeting. The participants will be informed that they at all times and without justification may withdraw their informed consent and choose no longer to participate in the study without any implications for their future treatment at Steno Diabetes Center Copenhagen. After signed informed consent, the study investigators, the regional ethical committee and other relevant authorities will have the possibility of accessing the medical records of the study participants in order to verify and monitor the project procedures and project data, provided confidentiality is kept. The participants are given a copy of the signed consent. Information on individual data and interpretation hereof will be communicated by the study dedicated doctor. The participant's right not to know their own data will be

respected. No study-specific tests are performed before informed consent for the study is obtained.

Publication plan

The study is initiated by Peter Rossing. Data is owned by the investigators. Positive, inconclusive as well as negative study results will be published in both national and international oral and written presentations as well as peer-reviewed international scientific journals. If data against expectations are not published in international journals, positive as well as negative study results will be published on a public website, for example www.clinicaltrials.gov.

Timeframe

Protocol finished	November 2019
Scientific Ethical Committee approval	December 2019
Inclusion of patients from	January 2020
Study completed (last participant, last visit)	January 2021
Data analysis (primary endpoint)	Spring 2021
Presentations	Autumn 2021
Publications	Winter 2021

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