Empagliflozin in type 2 diabetes with and without CKD and non-diabetic CKD: Protocol for 3 randomized, double-blind, placebo controlled cross-over trials -The SiRENA project

Steffen Flindt Nielsen, Camilla Lundgreen Duus, Niels Henrik Buus, Jesper Nørgaard Bech, Frank Holden Mose

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Abstract

Background: Sodium-Glucose-Cotransporter 2 inhibitors (SGLT2i) have revolutionized treatment of type 2 diabetes mellitus (DM2) and chronic kidney disease (CKD), reducing risk of cardiovascular and renal endpoints by up to 40%. The underlying mechanisms are not fully understood.

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Results: Recruitment began in April 2021 and was completed in September 2022. Examinations were completed by December 2022. 49 participants completed the project; 16 in the DM2 and preserved kidney function study, 17 in the DM2 and CKD study and 16 in the non-diabetic CKD study. Data analysis is ongoing. Results are yet to be published.

Conclusions: This article describes the rationale, design and methods used in a project consisting of three randomized, double-blind, placebo controlled cross over trials examining the effects of empagliflozin versus placebo in patients with DM2 with and without CKD and patients with non-diabetic CKD, respectively. Clinical Trial: EU Clinical Trials Register 2019-004303-12, 2019-004447-80 and 2019-004467-50

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Trial registration: EU Clinical Trials Register 2019-004303-12, 2019-004447-80 and 2019-004467-50

Keywords: SGLT2i; empagliflozin; renal function; blood flow; DM2; diabetes mellitus type 2; CKD; chronic kidney disease, vascular function

Introduction

The World Health Organization (WHO) has recently named diabetes the ninth leading cause of death globally [1]. Worldwide, more than 500 million people suffer from diabetes. By 2045 this number is expected to rise to more than 780 million [2]. The disease burden is immense and complications are common. One of the most common - and serious – complications is cardiovascular disease (CVD), which affects more than 30% of all patients with diabetes [3]. Other complications include retinopathy, neuropathy and nephropathy.

Chronic kidney disease (CKD) affects up to 50% of all patients with type 2 diabetes mellitus (DM2) [4, 5], and is common in patients without diabetes as well. It ranks just below diabetes as the 10th leading global cause of death, resulting in an estimated 1.3 million deaths annually. The most common cause of death in both diabetes and CKD patients is CVD [3, 6].

Recently, Sodium-Glucose-Cotransporter 2 inhibitors (SGLT2i) have revolutionized treatment of both DM2 and CKD. Originally developed as an anti-diabetic medication, SGLT2i block the SGLT2 channels in the proximal kidney tubule, inhibiting the reabsorption of sodium and glucose from the pre-urine, leading to glycosuria and a modest decrease in plasma glucose [7].
Several recent large randomized controlled clinical trials (RCTs) have shown that SGLT2i exert remarkable effects in patients with diabetes, both with and without CKD, reducing risk of death by CVD, hospitalization for heart failure and progression of CKD[8-10]. The effects are similar in patients with non-diabetic CKD, reducing risk of CKD progression and death by renal or cardiovascular causes by 30- 40%[11, 12]. As a consequence, current guidelines recommend treatment with SLGT2i for patients with DM2 both with and without concomitant CKD, as well as for patients with non-diabetic CKD [13, 14].

The mechanisms underlying these remarkable effects are, however, not yet fully understood. There are several possible pathways[15] – in this study, we examine the following four:

SLGT2i and renal hemodynamics

Increased renal reabsorption of sodium and glucose mediated by the SGLT2-channels is thought to be an important pathophysiological feature of diabetic nephropathy, instigating an increase in renal

blood flow (RBF) via tubule-glomerular feedback mechanisms[16]. This in turn causes renal hyper-filtration, intra-glomerular hypertension and, in time, kidney damage.
Intra-glomerular hypertension is not unique to diabetic nephropathy but is thought to play a key role in the pathophysiology of non-diabetic CKD as well. A decline in functioning nephrons leads to a cascade of maladaptive hemodynamic changes, including increased intra-glomerular pressure and hyper-filtration in the remaining nephrons[17, 18].

Conversely, by blocking the SLGT2-channel, SGLT2i are hypothesized to alleviate the changes by reducing RBF and hyper-filtration, which could be a possible explanation of the observed beneficial effects. The acute drop in estimated glomerular filtration rate (eGFR) seen after initiation of SGLT2i could to be a part of this mechanism as well[19]. While the effects on eGFR are well known, reduction in RBF has only been demonstrated in a single study in patients with type 1 diabetes as well as in animal models [20, 21]. None of the studies examining hemodynamic effects of SGLT2i in DM2 have found decreases in RBF [22, 23], and it has, to our knowledge, never been examined in patients with CKD.

SGLT2i and sodium balance

As well as blocking glucose reuptake, SGLT2i inhibit sodium reuptake in the proximal tubule, which leads to a transient increase in urinary sodium excretion [24]. Furthermore, a modest reduction in bodyweight, plasma volume and a decrease in sodium skin content is seen and may point to a decrease in volume status and total body sodium[25, 26]. This might help explain the rapid improvement in cardiac function seen after SGLT2i initiation[27]. However, the natriuretic effects of SGLT2i seem to dissipate quickly, possibly due to renal compensatory mechanisms[28]. These mechanisms are not yet fully understood and have been sparsely studied.

SGLT2i and vascular function

SGLT2i are also thought to exert an effect on the endothelial cells lining the inner walls of the blood vessels. DM2, CKD and especially the combination of the two are associated with endothelial dysfunction and subtle signs of endothelial dysfunction is often evident long before clinical signs of vascular damage [29, 30]. One of the most important reasons for endothelial dysfunction is decreased synthesis and bioavailability of nitric oxide (NO), which leads to a decrease in systemic vasodilation, dysfunctional cell adhesion, smooth muscle cell proliferation and hyper-coagulability[31, 32]. SGLT2i improve endothelial function in animal studies and seems to be able to improve vascular function and arterial stiffness in patients with DM [33-36]. So far, no studies have examined the effects in patients with CKD.

SGLT2i and the immune system

Inflammatory changes are common in the kidneys of CKD patients, and could be an important component in the development of glomerular injury, albuminuria and CKD progression [37]. The innate immune system is involved in CKD progression and could be a target for SGLT2i. Both pattern recognition molecules (e.g. collectin, mannan-binding lectine (MBL)) and complement activation pathways, notably the lectin pathway, could be involved in progression of CKD, particularly in diabetes. Therefore, it is of interest to examine this system in diabetic and non-diabetic CKD and determine sensitivity to SGLT2i. Complement system components like collectins, split products like anaphylatoxins C3a and C5a and terminal complexes (membrane attack complex (MAC)) can be measured in plasma and urine along with other markers of the immune system [38, 39]. SGLT2i can reduce expression of inflammatory molecules such as tumor necrosis factor α (TNF-α) and Interleukin 6(IL-6) [40]. Whether SGLT2i also affect markers of the immune system in

the kidney remains to be examined. Since complement system is associated with cell surfaces, we plan to employ urine microvesicles (exosomes) and use them as imprints or “wet biopsies” for apical membrane deposition of complement activation products along with quantitation of soluble components in plasma and urine.

Aims and hypotheses

We aim to examine the effects of the SGLT2i empagliflozin versus placebo on renal hemodynamics, vascular function, sodium balance and makers of the immune system in patients with DM2 with and without CKD, as well as in patients with non-diabetic CKD, hereby reflecting patient populations who would be offered SGLT2i in a clinical setting. In this article, we describe and discuss our research hypothesis and the methods we used.

We hypothesize that SGLT2i result in the following changes:

1) SLGT2i reduce RBF and glomerular filtration rate (GFR)
2) SGLT2i increase NO activity and improves endothelial function
3) SGLT2i increase fractional sodium excretion, which is modified by more distally localized compensatory mechanisms
4) SGLT2i increase renin angiotensin aldosterone system (RAAS) activity and decrease 24-hour ambulatory blood pressure and arterial stiffness
5) SLGT2i decrease renal innate immune activity

Methods

Design

We conducted three randomized, double-blind, placebo controlled cross over trials, each with identical study protocols but different study populations. We included patients with 1: DM2 and preserved kidney function, 2: DM2 and CKD and 3: non-diabetic CKD.

Each participant started with a run-in period of at least two weeks in which ongoing SGLT2i or non-steroidal anti-inflammatory drug (NSAID) treatment, which is known to affect both renal hemodynamics and fluid homeostasis[41], was paused. If patients were not treated with SGLT2i or NSAID prior to inclusion, they could proceed directly to randomization. If deemed necessary, SLGT2i could be substituted to a different class of anti-diabetic treatment. Substitution was done in accordance with national treatment guidelines at the time[42]. After run-in, participants were randomly assigned to four weeks of treatment with either empagliflozin 10 mg or matching placebo, both taken once daily. After a wash-out period of at least two weeks, participants were crossed over to four weeks of the opposite treatment. Each four-week treatment period was finalized with two examination days, one day at The University Clinic in Nephrology and Hypertension, Gødstrup Hospital, Denmark and one day at The Department of Renal Medicine, Aarhus University Hospital, Denmark.
We aimed to keep examination days adjacent, but due to logistic considerations we allowed for an interval of up to one week between examination days in each treatment period. If there was an interval between the examination days, the treatment period was extended concomitantly, ensuring that the last dose of study medication was taken on the morning of the last examination day (see Figure 1).

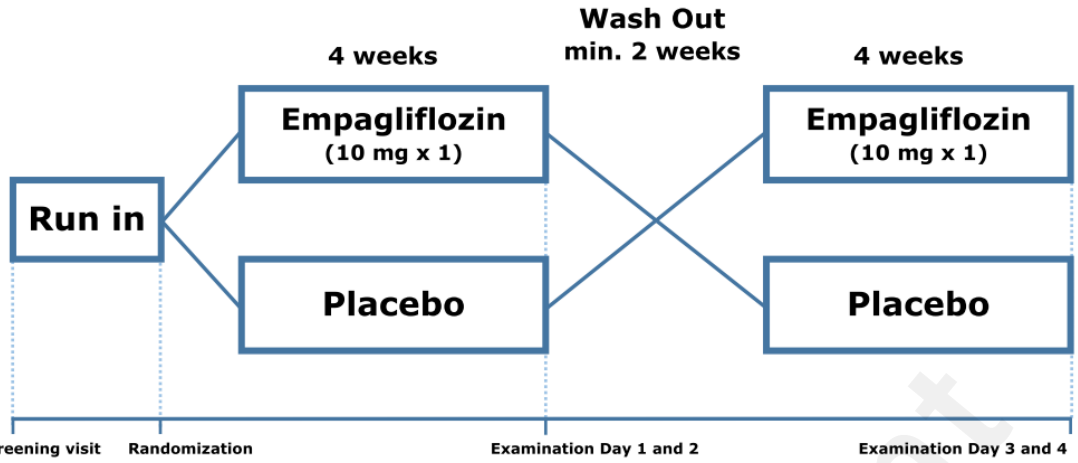

Figure 1 - Study design

Recruitment and screening

Participants were recruited through announcements at general practitioners, through newspaper advertisements and through e-mail or letters to participants from pervious trials, who has consented to being contacted about new trials. Furthermore, participants could be recruited from the outpatient clinics at The Department of Internal Medicine, Nephrology and The Department of Internal Medicine, Endocrinology, Gødstrup Hospital, Denmark. Informed and signed consent was obtained for all participants.

Participants were screened prior to inclusion to insure they fulfilled all inclusion criteria and none of the exclusion criteria. Screening involved physical examination, medical history, office blood pressure, electrocardiogram (ECG), urine test strip measurement, urinary albumin and albumin/creatinine ratio and the following blood samples: p-glucose, p-alanine transaminase (ALAT) b-glycated hemoglobin (HbA1c), p-potassium, p-sodium, p-albumin, p-creatinine, eGFR, B-leukocytes, B-hemoglobin, B-erythrocytes and B-thrombocytes. Office blood pressure was measured three times and calculated as an average of the last two measurements. It was measured in both arms and in case of difference between arms, the arm with the highest values were chosen for further measurements. If identical, the left arm was chosen.

Participants

Our power calculation was based on the primary end point, RBF. 15 patients were needed in each study to detect a minimal relevant difference in RBF of 0.167 ml/min/g, with a standard deviation (SD) of 0.180 ml/min/g, a two-sided α-level of 5% and a power of 90%.

Inclusion criteria:

Study 1 – DM2 and preserved kidney function

1) Age > 18 years.
2) eGFR > 60 ml/min/1.73 m^2.
3) DM2 diagnosed at least one year before inclusion and in stable medical anti-diabetic treatment for at least three months.
4) HbA1c 48-70 mmol/mol (DCCT values 6.5% - 8.6%).
5) Fertile women were to use safe contraception.

Study 2 – DM2 and CKD

1) Age > 18 years.

2) eGFR 20-60 ml/min/1.73 m^2.
3) DM2 diagnosed at least one year before inclusion and in stable medical anti-diabetic treatment for at least three months.
4) HbA1c 48-70 mmol/mol. (DCCT values 6.5% - 8.6%).
5) Fertile women were to use safe contraception.

Study 3 – Non-diabetic CKD

1) Age > 18 years.
2) eGFR 20-60 ml/min/1.73 m^2.
3) Fertile women were to use safe contraception.

Exclusion criteria:

Study 1 and 2 – DM2 and preserved kidney function and DM2 and CKD

1) Type 1 diabetes.
2) Alcohol or substance abuse.
3) Pregnancy or breast feeding.
4) Anamnestic or clinical signs of heart- or liver failure.
5) Active cancers, aside from skin cancers (spinocellular or basocellular carcinomas)
6) Body mass index (BMI) > 35 kg/$m^{2.}$
7) Allergies or unacceptable side effects to the experimental treatment or background treatment.
8) If the investigator found the participant unfit to complete the trial.
9)Previous kidney transplant.
10)Autosomal Dominant Polycystic Kidney Disease (ADPKD).

Study 3 – Non-diabetic CKD

1)Same as in study 1 and 2
2) Type 2 diabetes mellitus

Withdrawal criteria:

For all three studies, participants were withdrawn if they developed an exclusion criterion, withdrew consent, were non-compliant or experienced serious or unacceptable adverse events.

Randomization

Randomization numbers were provided by the manufacturer, Boehringer-Ingelheim (Boehringer-Ingelheim, Ingelheim am Rhein, Germany). Randomization was performed by the Hospital Pharmacy, Central Denmark Region, Department Gødstrup. Participants were randomized in blocks of four. Treatment assignment and allocation were concealed from clinicians, participants and research staff until the trials were completed and as long as they were involved in data analysis. A copy of the randomization list and sealed envelopes with individual randomization numbers were kept in a locked safe at The University Clinic of Nephrology and Hypertension, in case un-blinding was required. A single participant could be un-blinded without it affecting the rest of the trial. At the end of the project the envelopes will be returned to the Hospital Pharmacy where a receipt will be drawn up

Study Medication

The active treatment, empagliflozin 10 mg, and matching placebo was produced and distributed by

the manufacturer, Boehringer-Ingelheim. The placebo tablet was identical to empagliflozin in every way, except for the lack of active substance. Study medication was delivered from The Hospital Pharmacy in identical pill bottles and were allocated corresponding to the randomization number.
Compliance was checked with a phone call midway through each examination period and by pill count when the study medication was returned on the last examination day.

Endpoints

Primary endpoint:

- Renal blood flow (RBF)

Secondary endpoints:

- GFR
- Renal vascular resistance (RVR), Filtration Fraction (FF), Afferent and Efferent Arteriolar Resistance (R_a and R_e)
- Vascular function
- The NO-system, measured as plasma and urinary levels of nitrite, nitrate and cyclic guanosine monophosphate (cGMP)
- The complement system, measured as plasma and urine levels of MBL, collectin kidney 1 (CL-K1), collectin liver 1 (CL-L1), mannan-binding lectin serine protease (MASP) 1-3, C4c, C3c, C3dg and sC5b-9 and urinary exosomes
- Systemic hemodynamics, measured as 24-hour ambulatory brachial blood pressure, heart rate, pulse wave velocity (PWV) and peripheral resistance.
- Plasma levels of renin, angiotensin II, aldosterone, vasopressin and brain natriuretic peptide (BNP)
- Water and sodium excretion: urinary sodium, free water clearance, urinary glucose, urinary albumin, fractional sodium excretion, urinary excretion of tubular transporter proteins (aquaporin 2 (AQP2), endothelial sodium channel (ENaC), sodium chloride symporter channel (NCC) and sodium-potassium-chloride cotransporter (NKCC)), Extracellular Body Water (EBW), Total Body Water (TBW), Intracellular Body Water (IBW), Adipose Tissue Mass, Erythrocyte Salt Sensitivity (ESS)
- HbA1c and p-glucose
- β-hydroxy butyrate and urate
- Urinary excretion of adenosine, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and IL-6
- Plasma concentrations of parathyroid hormone (PTH), phosphate, calcium, alkali phosphatase and fibroblast growth factor (FGF23) and urinary excretion of phosphate and calcium.

Study Methods

^{82}Rb-PET/CT

RBF was measured with ^{82}Rubidium positron emission tomography/ computed tomography (^{82}Rb-PET/CT scans). All scans were performed on a Siemens Biograph mCT; 64 slice-4R. (Siemens Healthcare GmbH, Erlangen, Germany). The method has been previously described by Langaa[43, 44].
Participants rested in a sitting position for at least 30 minutes prior to the scan. During the scan, participants were placed in supine position with arms rested over their head. After positioning, a low dose CT-scan (25 mAs, 100 kV) was performed for attenuation control. This was immediately followed by a bolus injection of 555 MBq ^{82}Rb through a peripheral venous catheter (PVC), where after an eight-minute dynamic PET-scan was performed. Through iterative reconstruction, 3D images

of the activity changes in the abdominal aorta and the parenchyma of both kidneys were generated.
A 1-tissue compartment model was used for flow estimation, and RBF was calculated as a K_1-value based on activity uptake in the abdominal aorta and both kidneys. Values were calculated using PMOD© (PMOD Technologies Ltd., Zürich, Switzerland).
^{82}Rb was obtained using an ^{82}Rb-generator (Cardiogen-82; Bracco Diagnostics Inc., Monroe Township NJ, USA). Scans were done in cooperation with The Department of Nuclear Medicine, Regional Hospital Gødstrup.

Venous occlusion strain gauge plethysmography

Vasodilatory function was measured using classic forearm venous occlusion plethysmography (Hokanson EC6; Bellevue, WA, USA) as previously described by Fredslund [45], though we did not perform measurements in the contralateral arm. An indium-gallium strain gauge placed around the forearm senses changes in forearm volume. Changes in forearm volume during brief, very fast, occlusions (using the Hokanson E20 inflator) of venous outflow by a cuff on the upper arm, will then reflect the arterial inflow. The plethysmography method therefore allows direct assessment of the effects of vasoactive drugs infused into the brachial artery. In the present project we used infusion of acetylcholine (ACh) and sodium nitroprusside (SNP) for evaluation of endothelium dependent and independent vasodilatation, respectively.

GFR

GFR was determined through clearance of ^{99m}Technetium- diethylene-triamine-pentaacetate (^{99m}Tc-DTPA). 25 MBq of ^{99m}Tc-DTPA was injected intra-venously through a PVC. Before injection, a zero sample was drawn. Blood samples were drawn after three, four and five hours, measuring residual plasma ^{99m}Tc-DTPA activity, whereby GFR was calculated. Measurements were done in cooperation with The Department of Nuclear Medicine, Regional Hospital Gødstrup.

Blood pressure measurement and arterial stiffness

24-hour ambulatory brachial blood pressure, heart rate, PWV and arterial stiffness was measured with Mobil-O-Graph® (I.E.M. GmbH., Aachen, Germany)

Biochemical analyses

Plasma and serum levels of sodium, HbA1c, glucose, BNP, potassium, albumin, creatinine, phosphate, PTH, alkali phosphatase, urate, total protein, hemoglobin, erythrocyte volume fraction (EVF), thrombocytes and calcium were routinely analyzed by The Department of Biochemistry, Gødstrup Hospital, Denmark. β-hydroxy butyrate was measured with a FreeStyle Precision Neo® point of care devise (Abbott Laboratories, Chicago, Illinois, USA)

Plasma levels of renin, angiotensin II, aldosterone, and vasopressin were measured by radioimmunoassay. Plasma levels of cGMP and FGF23 measured by ELISA.
Plasma and urine levels of nitrite and nitrate were measured by spectrophotometry.
ESS was measured with salt blood test (CARE Diagnostica Laborreagenzien GmbH., Voerde (Niederrhein), Germany). Plasma and urinary osmolality were measured by freeze point depression with an Advanced Instruments A2O osmometer (Advanced Instruments, Norwood, MA, USA)

Plasma and urinary levels of MBL, CL-K1, CL-L1, MASP-1-3, C4c, C3c, C3dg, sC5b-9 CL-K1 and CL-L1 and urinary exosomes were measured at The Department of Cardiovascular and Renal Research, University of Southern Denmark.

Urine volume and urinary levels of sodium, creatinine, albumin, calcium and phosphate were

measured routinely by The Department of Biochemistry, Gødstrup Hospital, Denmark.
Urinary levels of glucose, AQP2, ENaC, NCC and NKCC were measured by radioimmunoassay, cGMP, IL6, NGAL, KIM-1 and adenosine were measured by ELISA.

Bio-impedance measurement

EBW, TBW, IBW and Adipose Tissue Mass were measured with bio-impedance spectroscopy using Body Composition Monitor (BCM) (Fresenius Medical Care AG & Co. KGaA, Bad Homburg, Germany)

Renal Hemodynamics

RVR was calculated as Mean Arterial Pressure (MAP)/ RBF. FF was calculated as GFR/ Renal Plasma Flow. R_a and R_e was calculated using the Gomez equations [46].

Statistics

Data following a normal distribution was calculated with parametric statistics. Paired data was compared with either paired t-test or ANOVA. Non-parametric statistics were applied if variables were not normally disrupted. Paired comparisons were compared with Wilcoxon Signed Rank test or Friedman's' test. Statistics were performed using STATA 18.0 (StataCorp LLC, College Station, Texas, US).

Experimental procedure

Prior to examination

Fluid intake was standardized from four days prior to the first examination day till the last examination day in each treatment period. Each participant was encouraged to drink at least 2 liters of water per day. Two cups of coffee or tea daily was allowed, except for 8 hours prior to the examinations. Alcohol or soft drinks were prohibited in this period. The use of mouthwash products was prohibited throughout the study period. Furthermore, in each treatment period participants were encouraged to adhere to their usual diets from 4 days prior to the first examination and till the last examination. Participants were not required to fast prior to examination but did not eat throughout the examinations.

Examination day – University Clinic in Nephrology and Hypertension, Gødstrup Hospital

Participants were set to meet at 08:00, having ingested their usual medication and the study medication. First, participants emptied their bladder, body weight was measured and they rested in a chair for 30 minutes. A pregnancy test was performed in fertile women. Then, a PVC was inserted and the Mobil-O-Graph was placed on the upper arm, opposite the PVC. At time point 0 blood samples were drawn and a bolus injection of ^{99m}Tc-DTPA was given (Figure 2). After this, the ^{82}Rb-PET/CT scan was performed. Two successive BCM measurements were done within the first two hours of the examination day.
Urine was collected at two and five hours and if additional voiding was necessary. All urine, except the first void, was pooled and analyzed. 50 mL fresh spot urine was collected from the first void, a protease inhibitor was added, and the sample was frozen for later exosome analyses. Two mL spot urine, also from the first void was frozen for analysis of complement factors. Blood samples were drawn at three, four and five hours. At the end of the examination day body weight was measured again. The Mobil-O-Graph was removed by the participant 24 hours after mounting. During the entire examination day, participants were to rest in a bed or on a chair. Voiding was done standing or sitting. Participants were given 175 ml of water each hour from time point 0 till the end of the

examination day.

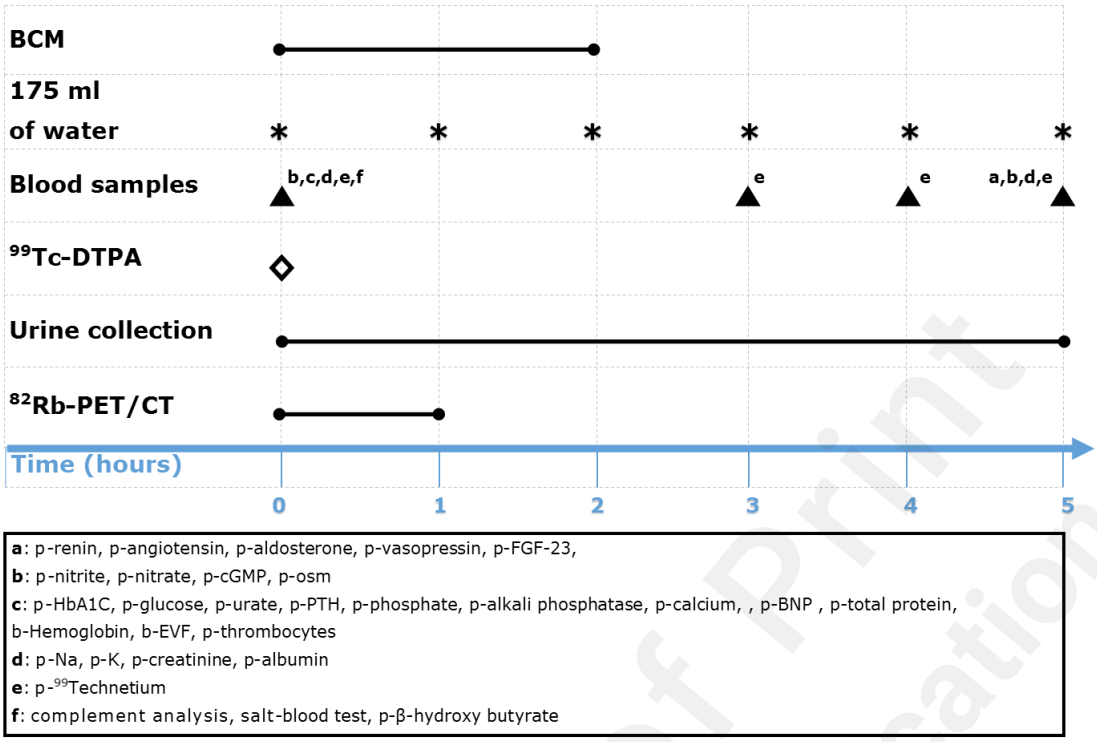

Figure 2 - Schematic illustration of the examination day at Gødstrup Hospital

Examination day – Department of Renal Medicine, Aarhus University Hospital

The strain gauge plethysmography was performed at the Department of Renal Medicine, Aarhus University Hospital (Figure 3 and Figure 4). Participants had taken their usual medication and the study medication on the morning prior to the examination. Participants were placed in supine position in a room where the temperature was kept fixed at 25°C. The brachial artery was then cannulated with a 27G needle and kept from clotting by a slow infusion of isotonic saline. The strain gauge was placed on the broadest part of the forearm, a venous occlusion cuff was placed on the upper arm and an arterial occlusion cuff was placed at the wrist. After 30 minutes of saline infusion, baseline measurements were recorded (eight readings were performed per measurement). ACh was infused in increasing concentrations at five-minute intervals. Measurements were recorded at each concentration. After the last measurement, saline was infused for another 30 minutes. Afterwards isotonic glucose was infused for 5 minutes, and new baseline measurements were recorded. SNP was now infused in increasing concentrations again at five-minute intervals, with measurements done at each concentration. Arterial circulation to the hand was interrupted during infusion. When the last measurement had been performed infusion was stopped, the needle was removed, and the examination day ended.

Due to delivery issues, ACh was replaced with carbachol (CCh) after the first 23 participants had been examined. Two participants, both in the DM and CKD group, had their first examination done with ACh and the second with CCh. The remaining examinations were done entirely with CCh.

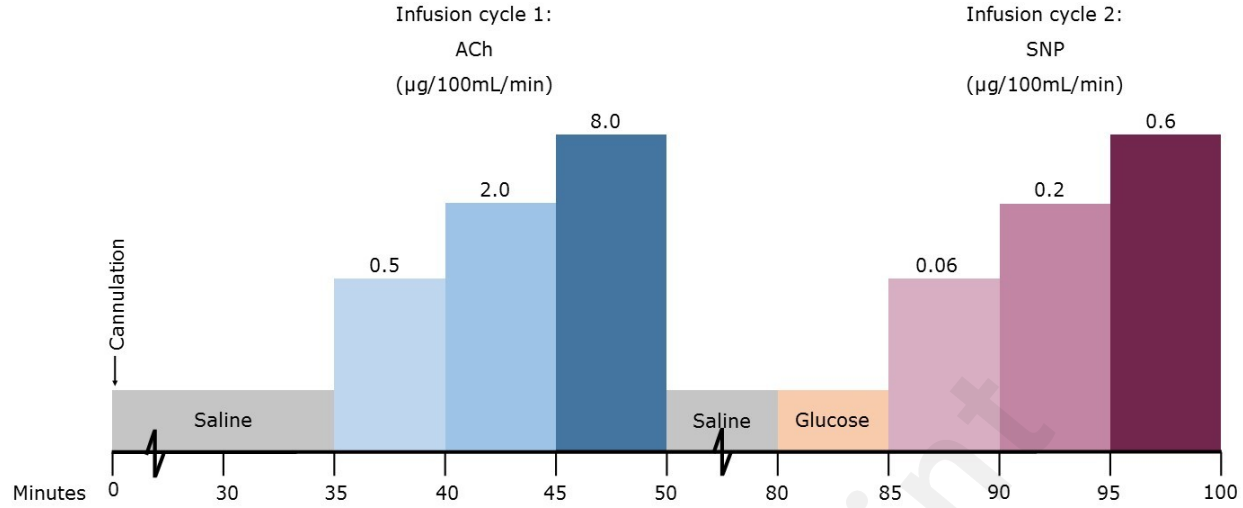

Figure 3 - Schematic timetable of the strain gauge plethysmography experimental protocol using ACh

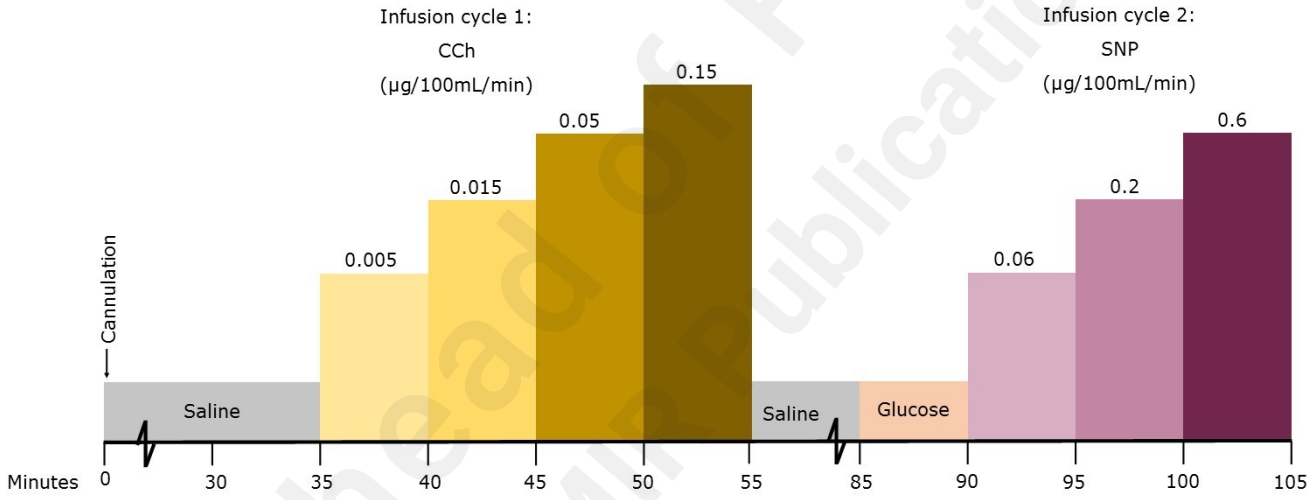

Figure 4 - Schematic timetable of the strain gauge plethysmography experimental protocol using CCh

Ethics

All three studies were approved by The Central Denmark Region Committees on Health Research Ethics (case numbers: 1-10-72-214-20, 1-10-72-339-20 and 1-10-72-340-20, respectively) and The Danish Medicines Agency (EU Clinical Trials Register: 2019-004303-12, 2019-004447-80 and 2019-004467-50) and were conducted in accordance with the Declaration of Helsinki 2013. Informed and signed consent was obtained for all participants. The study was monitored by the Good Clinical Practice (GCP) Unit of Aarhus and Aalborg University Hospitals. All data has been de-identified. Participants could be compensated for travel expenses related to the studies.

Results

Approval from the regulatory agencies were obtained for the first study (DM2 and preserved kidney function) by October 2020. Recruitment began in April 2021. Approval for the remaining studies was obtained by May 2021 and recruitment began in March 2022. Inclusion of participants for all studies was completed by September 2022. Examinations started in August 2021 and were completed (last patient, last visit) by December 2022. 49 participants completed the project; 16 in the DM2 and

preserved kidney function study, 17 in the DM2 and CKD study and 16 in the non-diabetic CKD study. Data analysis is currently on-going. So far, no results from the project have been published.

Discussion

In this article, we present the background, hypothesis, design, and methodology of a project consisting of three RCTs examining the effects of the SGLT2-inhibitor empagliflozin versus placebo on a wide range of parameters in different patient populations. This will hopefully provide important information on the mechanisms underlying the beneficial effects of SGLT2i and is the first project to examine both patients with and without diabetes and with and without CKD using the same experimental set up. To our knowledge, very few mechanistic studies have examined SGLT2i in a CKD population.

We employ a novel method for estimating RBF by using ^{82}Rb-PET/CT. Compared to measuring effective renal plasma flow (ERPF) with para-aminohippuric acid (PAH) clearance, a method often employed in other studies examining hemodynamic effects of SGLT2i [20, 23, 47], flow estimation with ^{82}Rb-PET/CT can be performed much quicker (in less than half an hour) without the need for blood or urine samples and allows for estimation of single kidney RBF. Furthermore, the effective radiation dose of a single scan is limited (~1 mSv). There is an ongoing discussion whether our method in fact reflects RBF or rather RPF. This should be taken into account when calculating intra-renal hemodynamics since the calculations rely on which parameter is used. However the method has proven both precise and reliable when evaluating relative changes in kidney perfusion, which is what we assess in this project[43, 44].

Vascular function and SGLT2i have been studied widely, as specified in the introduction, though mainly by flow mediated dilation (FMD)[34, 35]. The venous occlusion strain-gauge plethysmography gives a more in-depth examination of the potential mechanisms at play by measuring both endothelial dependent and independent vasodilation.

A clear strength of our project is the robust, randomized, placebo controlled cross over design. We examine different patient populations using the exact same design, allowing for comparison of effect between groups. Furthermore, we investigate a number of different variables, allowing for different mechanisms to be examined. With the current updated guidelines, all three examined groups represent patient populations who would be offered SGLT2i in a clinical setting, which adds to the generalizability of our results. It is important to note that inclusion in the DM2 and CKD group did not require a diagnosis of diabetic nephropathy, so participants could potentially have kidney disease of other etiologies. We used empagliflozin 10 mg and not the 25 mg dose, since this was the dose used in the EMPA-kidney study and since both doses have equivalent effects on both renal outcomes and eGFR-decline[8].

Limitations

Our project has several limitations; one being small study sample sizes, increasing the risk of the studies being underpowered for detecting changes in secondary endpoints. As well as a strength, examining multiple mechanisms of action can be a limitation, since it increases the risk of type 1 errors, thus most of our secondary endpoints should be interpreted as exploratory. Our inclusion criteria are broad by design, mainly to reflect a real-world patient population that could potentially be prescribed SGLT2i, but the broad criteria make for a more heterogeneous study population and increases the risk of heterogeneity of the outcome effects. Despite being a well-known risk-factor for disease progression in both DM2 and CKD[48], we did not make albuminuria an inclusion criterion. We chose this approach because albuminuria was not an inclusion criterion for all patients in neither the EMPA-REG OUTCOME, DECLARE-TIMI 58 or EMPA-KIDNEY trial[49-51], although it was in the CREDENCE and DAPA-CKD trials[9, 12]. By allowing inclusion of patients without albuminuria, we risk including patients at low risk where treatment benefits could be less

pronounced. Furthermore, patients were not fasting before examinations, nor did we take steps to ensure stable blood glucose levels throughout the examination days. This was considered, but discarded for feasibility reason, but it does increase the risk of our outcomes being affected by confounders. Adding to this was the fact that we allowed for substitution of anti-diabetic treatment in the run-in period if SGLT2i were paused. This was done to ensure glycemic control during the trial period, but may have introduced further confounding. This methods article has been written after data collection was completed. While it would have been optimal to publish it prior to inclusion, we judge this to be a precise description of the methods used and it will provide a valuable framework for future articles, detailing exactly how our results were obtained and what considerations lay behind them.

Conclusions

This article describes the rationale, design and method used in a project consisting of three randomized, double-blind, placebo controlled cross over trials, examining the effects of empagliflozin versus placebo in patients with DM2 with and without CKD and patients with non-diabetic CKD, respectively.

Author contributions

All authors contributed to the manuscript. SFN, JNB, NHB and FHM designed the project. SFN drafted the manuscript. SFN, CLD, JNB, NHB and FHM edited the manuscript. All authors approved the final manuscript.
Generative artificial intelligence was not used in any portion of the manuscript.

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Boehringer-Ingelheim was given the opportunity to review the manuscript for medical and scientific accuracy as it relates to Boehringer-Ingelheim substances, as well as intellectual property considerations. Boehringer-Ingelheim had no role in the design of the study nor will they have a role in the analysis and interpretation of results.

Conflicts of Interest

FHM disclosed advisory board participation and speaker honoraria from Boehringer-Ingelheim and AstraZeneca. JNB disclosed advisory board participation for AstraZeneca, Boehringer and Bayer. SFN, CLD and NHB have no conflicts of interest to declare.

Abbreviations

^{82}Rb: ^{82}Rubidium
^{99m}Tc: ^{99m}Technetium
ACh: acetylcholine
ALAT: alanine transaminase
ANOVA: analysis of variance
AQP2: aquaporin 2
BCM: body composition monitor

BMI: body mass index
BNP: brain natriuretic peptide
CCh: carbachol
cGMP: cyclic guanosine monophosphate
CKD: chronic kidney disease
CL-K1: collectin kidney 1
CL-L1: collectin liver 1
CT: computer tomography
CVD: cardiovascular disease
DM: diabetes mellitus
DM2: diabetes mellitus type 2
DTPA: diethylene-triamine-pentaacetate
EBW: extracellular body water
ECG: electrocardiogram
eGFR: estimated glomerular filtration rate
ELISA: enzyme-linked immunosorbent assay
ENac: epithelial sodium channel
ERPF: effective renal plasma flow
ESS: erythrocyte salt sensitivity
EVF: erythrocyte volume fraction
FF: filtration fraction
FGF23: fibroblast growth factor 23
FMD: flow mediated dilation
GFR: glomerular filtration rate
HbA1c: hemoglobin A1c
IBW: intracellular body water
IL-6: interleukin 6
KIM-1: kidney injury Molecule-1
MAP: mean arterial pressure
MASP: mannan-binding lectin serine protease
MBL: mannan-binding lectine
MBq: megabecquerel
mSv: mili-Sievert
NCC: sodium chloride symporter channel
NGAL: neutrophil gelatinase-associated lipocalin
NKCC: sodium-potassium-chloride cotransporter
NO: nitric oxide
NSAID: non-steroidal anti-inflammatory drugs
PAH: para-aminohippuric acid
PET: positron emission tomography
PTH: parathyroid hormone
PVC: peripheral venous catheter
PWV: pulse wave velocity
R_a: afferent arteriolar resistance
RAAS: renin angiotensin aldosterone system
RBF: renal blood flow
RCT: randomized, controlled trial
R_e: efferent arteriolar resistance
RVR: renal vascular resistance
SD: standard deviation

SGLT2i: sodium-glucose-cotransporter 2 inhibitors
SNP: sodium nitroprusside
TBW: total body water
TNF-α: tumor necrosis factor-α
WHO: World Health Organization

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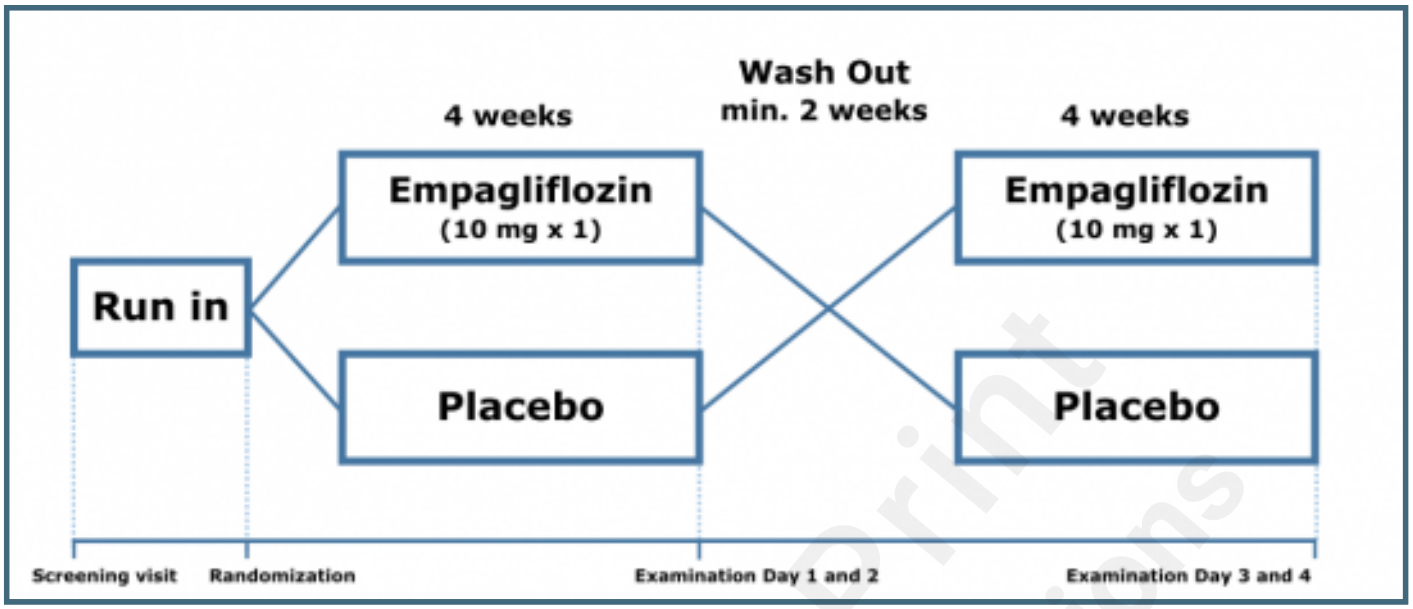
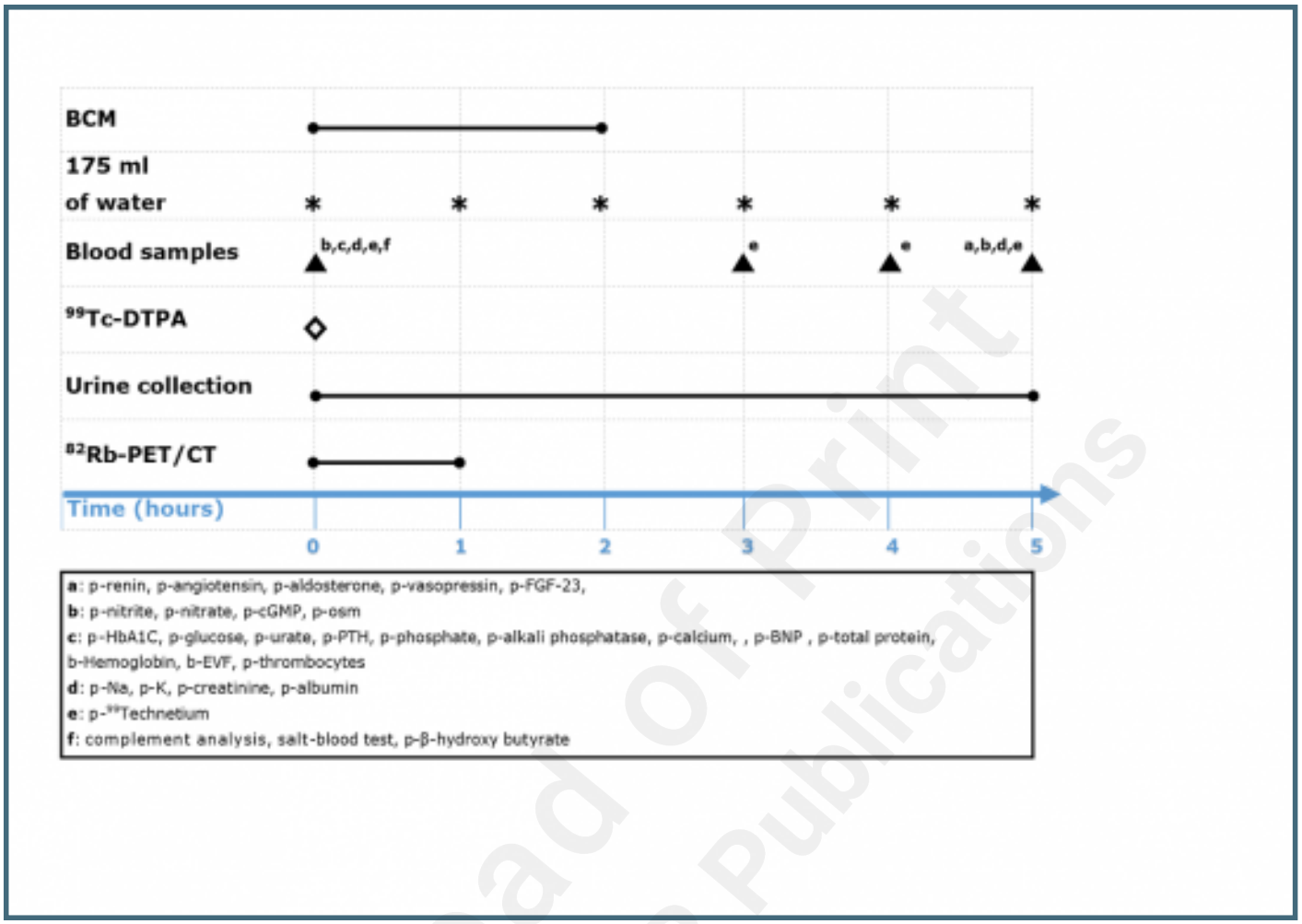
Supplementary Files

Figures

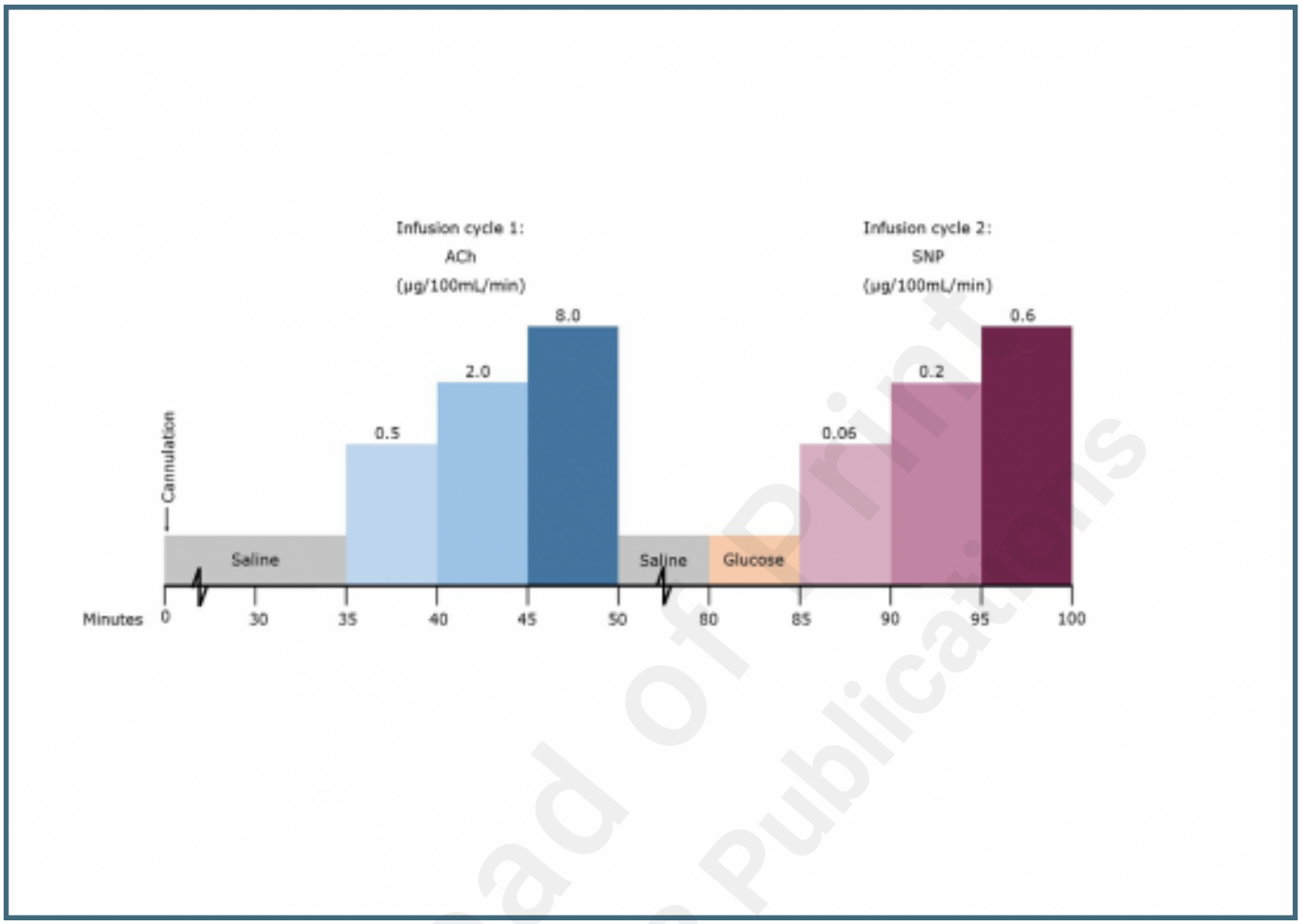
Study design.



Schematic illustration of the examination day at Gødstrup Hospital.



Schematic timetable of the strain gauge plethysmography experimental protocol using ACh.



Schematic timetable of the strain gauge plethysmography experimental protocol using CCh.