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Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

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ABSTRACT

Introduction: A diagnosis of type 2 diabetes (T2D) more than doubles the risk of cardiovascular disease (CVD), with heart failure (HF) being one of the most common complications with a severe prognosis. The landmark EMPA-REG study demonstrated that treatment with the sodium glucose cotransporter-2 (SGLT-2) inhibitor empagliflozin rapidly and significantly reduces CVD mortality and admission rates for HF. However, the mechanisms behind this reduction in clinical events are unknown.

The current study was designed to investigate the effects of the SGLT-2 inhibitor empagliflozin on myocardial perfusion and function in patients with T2D and high CVD risk.

Methods and analysis: In this investigator-initiated, randomized, double-blind controlled clinical trial, 92 patients with T2D and established CVD or high CVD risk, will be randomized to treatment with empagliflozin 25 mg or a matching placebo for 13 weeks. The primary outcome measure is change in myocardial flow reserve measured quantitatively by Rubidium-82 position emission tomography (^{82}Rb-PET). In a substudy, invasive hemodynamics at rest and during exercise will be measured at baseline and following the intervention, using right heart catheterization.

Ethics and dissemination: The study protocol (v7, 02/08/2018) has been approved by the Ethics Committee of the Capital Region, Danish Data Protection Board and the Danish Medicines Agency, and it will be monitored according to the Good Clinical Practice (GCP) regulations from the International Conference on Harmonization.

Trial Registration: ClinicalTrials.gov Identifier: NCT03151343

ARTICLE SUMMARY

Strengths and limitations of this study:

- Double-blinded, randomized and placebo controlled.

- The use of advanced imaging techniques.
- Single-center
- No hard endpoints, all outcomes are based on surrogates

INTRODUCTION

Type 2 diabetes (T2D) is a significant risk factor for cardiovascular disease (CVD), including heart failure (HF), and improved glycaemic control is only modestly beneficial in reducing macrovascular disease.[1]

In 2008, prompted by concerns that the T2D drug rosiglitazone might increase the risk of myocardial infarction, the U.S. Food & Drug Administration published guidelines effectively mandating large cardiovascular outcome trials for new T2D drugs. Several such trials have been completed or are ongoing. The trials have primarily examined the safety of dipeptidyl peptidase 4 (DPP-IV) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RA) and sodium–glucose cotransporter 2 (SGLT2) inhibitors.[2–8] The DPP-IV inhibitor trial SAVOR-TIMI indicated an increase in the secondary endpoint, hospitalization due to HF with the active comparator, while another DPP-IV trial, TECOS, as well as the newly published CARMELINA did not demonstrate an increased risk with the active drug. The GLP-1 RA trials LEADER and SUSTAIN although with a reduction in the primary MACE endpoint were neutral with regards to risk of HF. Most of the SGLT-2 trials have demonstrated CV benefits[9] via a reduction of the primary endpoint MACE. In the EMPA-REG outcome study, the SGLT-2 inhibitor empagliflozin reduced the risk of CV death by 38% and in contrast to the GLP-1 RA trials, admission due to HF by 35% in a high-risk T2D population.[6]

The novel evidence from the SGLT-2 inhibitor trials was surprising, because the treatments only had a modest effect on classical CV risk factors. Indeed, the mechanism behind their profound effect on CV death and risk of HF remains unknown, although several hypotheses have been proposed. An effect reducing the atherosclerotic burden is considered unlikely, because the risk of CV events was reduced within weeks of treatment. Thus, the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is considered to play a significant role in T2D-related CVD[10] as well as in HF with preserved ejection fraction.[11] Furthermore, coronary vascular dysfunction, particularly decreased vasodilator capacity, is a significant predictor for CV death in patients with T2D.[12,13] Notably, T2D patients with preserved coronary vasodilation had the same low risk of CVD as subjects without diabetes[12]. Experimental studies have shown that SGLT-2 inhibitor treatment significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary arterial fibrosis and improves vasodilatation in diabetic rodents.[14,15] Thus, part of the underlying effect of empagliflozin in EMPA-REG could be driven by improvement in myocardial microcirculation.

A complementary hypothesis has been suggested to explain the early beneficial effects on HF hospitalization found in the EMPA-REG trial.[16] Inhibition of SGLT-2 is associated with increased diuresis, thus the sodium- and volume-reducing effect could favour cardiac loading conditions in patients with T2D with subclinical dysfunctions of the heart. The proposed effects on central hemodynamics, especially right and left heart filling pressures and cardiac output, can be measured directly by right heart catherization. Pulmonary capillary wedge pressure (PCWP), a measure of left heart filling pressure, has previously been shown to be associated with functional capacity among patients with HF.[17] Therefore, investigating the effect of SGLT-2 inhibitor on central hemodynamics during rest and exercise may contribute vital knowledge on the mechanisms behind the markedly reduced risk of admission for HF demonstrated in the EMPA-REG and DECLAIRE TIMI trials[6,8].

The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR) and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac function.[18] Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral glucose uptake[19] through a reduction in glucose toxicity. Moreover they induce a small increase in plasma ketone bodies, which may serve as a preferred fuel source for the myocardium.[20] Therefore, further knowledge on the effects of SGLT-2 inhibitors on metabolic parameters, including dysfunctional adipose tissue and the relationship with parameters on cardiac function in patients with T2D is warranted.

In summary, SGLT2 inhibitors have been shown to reduce CVD in high-risk T2D patients, with several explanatory hypotheses being suggested that remain to be tested in clinical trials.

Hypothesis

Treatment with empagliflozin for 13 weeks improves the myocardial flow reserve (MFR) in patients with T2D and high cardiovascular risk.

Objectives

The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by ^{82}Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study, the effect of empagliflozin on key hemodynamic parameters will be measured during right heart catherization at rest and during exercise. Key secondary outcomes include changes in cardiac echocardiographic evaluations of systolic and diastolic functions, functional capacity by accelerometry, and changes in glucose metabolism, plasma ketone bodies and adipose tissue function.

METHODS AND ANALYSIS

Trial design

This is an investigator-initiated, randomized, placebo-controlled, double-blind trial. Participants will be recruited from the T2D and cardiology outpatient clinics at Herlev-Gentofte University Hospital, Steno Diabetes Center Copenhagen and Rigshospitalet, Denmark.

Study population

The study will include 92 patients with T2D who have either additional CV risk factors or pre-existing CVD. Detailed inclusion and exclusion criteria are listed in Table 1. Eligible patients will be invited for screening, and patients who meet all the criteria will be randomized to the double-blinded treatment. Participants will be considered part of the intention to treat (ITT) group after receiving the first dose of the study medication"

Trial Intervention

Participants will be randomized 1:1 to either empagliflozin 25 mg or matching placebo once daily for 13 weeks. The trial medication will be randomized in computer-generated 1:1 blocks of 10 by the study pharmacy (Glostrup Pharmacy, Copenhagen, Denmark). Participants will receive randomization numbers and the corresponding medication containers sequentially. In case of medical emergency, participants can be unblinded individually.

Trial visits and procedures

Visits

A schematic overview of the trial visits is presented in Table 2. At the screening visit (V_0), informed consent will be obtained, and patients will be assessed for eligibility based on medical histories, physical examinations and blood samples. Ineligible participants will be counted as screen failures, and the reason for screen failure will be recorded.

Outcome-related procedures are performed at the baseline (V_1) and after 13 weeks of treatment (V_3). The trial medication will be dispensed when all the procedures pertaining to V_1 have been completed.

After 3 to 6 weeks of treatment, the participants will visit the trial site for blood tests and a physical examination (V_2). Participant status and compliance as well as adverse events will be assessed at V_2, V_3 and during two phone calls (T_1 and T_2).

^{82}Rb-PET

Myocardial perfusion and myocardial flow reserve (MFR) will be measured using cardiac ^{82}Rb-PET, which allows for flow quantification in absolute terms. To determine the MFR, measurements will be performed at rest and during adenosine-induced stress. A standard clinical protocol will be used. Participants will be scanned in the supine position using a Siemens Biograph mCT/PET 128-slice scanner (Siemens Medical Solutions, Knoxville, USA). Low-dose non-contrast CT will be acquired for attenuation correction as well as measurement of cardiac adipose tissue volume. Approximately 1100 MBq of ^{82}Rb will be obtained from a CardioGen-82 Sr-82/Rb-82 generator (Bracco Diagnostics, Inc., Princeton, New Jersey, USA) and intravenously infused with a constant flowrate of 50 mL/min. List-mode 3D data acquisition will begin with the tracer infusion and continue for 7 minutes. Static and ECG-gated images will be reconstructed with a 2.5-minute delay to allow ^{82}Rb to clear from the blood pool. Maximal hyperaemia will be induced with adenosine infused at 140 µg/kg/min for 6 minutes. After 2.5 minutes of adenosine infusion, intravenous ^{82}Rb infusion and list-mode acquisition will follow the same protocol as for rest. Myocardial blood perfusion quantification (in mL/min/g) will be performed using Cedars-Sinai QGS+QPS 2015.6 software (Cedars-Sinai Medical Center), which is based on a single-compartment model for Rb-82 tracer kinetics.

Hemodynamics sub-study

In a substudy, 38 participants randomly selected from the primary study population will perform a graded exercise test until maximal exertion using a supine ergometer with simultaneous invasive hemodynamic measurements. Using local anaesthesia, A Swan-Ganz catheter will be placed into the pulmonary artery via the right internal jugular vein. The following hemodynamic variables will be assessed: pulmonary capillary wedge pressure (PCWP), cardiac output (CO) using thermodilution, central venous pressure (CVP and pulmonary artery pressure (PAP). Following measurements at rest, participants will be transferred to a supine bicycle ergometer, and the measurements will be repeated (leg-raise situation). The participant will be instructed to pedal at 60 rpm and the workload is incrementally increased at steps of 10watts. Measurements during exercise will be obtained at 25 watts and during peak exercise. Blood sampled from the pulmonary vein will be obtained at rest, 25 watts and at peak exercise for analyses of lactate, oxygen saturation and other blood gas variables.

Echocardiography

Echocardiographic measurements will be obtained using 3D and 2D imaging at rest: left ventricular ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric

relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a') velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal speckle tracking) will be assessed via 2D echocardiography.

Cr-51 EDTA

The Cr-51 EDTA method will be used to measure GFR. A single intravenous administration of 3.7MBq of Cr-51 EDTA is given. A venous sample is drawn four hours after administration.

Accelerometry

A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the accelerometer continuously for 7 days, except for bathing and swimming.

Ambulatory blood pressure

24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every hour during the night.

Adipose tissue biopsy

Biopsies will be obtained from the abdominal subcutaneous tissue during a fasting state using the Bergstrom needle technique. Total mRNA will be extracted from adipose tissue with commercially available lipid tissue kits to measure mRNA expression of adipocytokines. Quantification of inflammatory cells will be performed according to validated histological procedures.

Oral Glucose Tolerance Test (OGTT)

The fasting participant will drink a solution of 75g of glucose. Blood will be drawn at time points 0, 30, 60 and 120 minutes, and measurements of plasma glucose, C-peptide and plasma insulin will be taken.

Questionnaires

Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.

Heart rate variability (HRV)

Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and while performing the Valsalva maneuver.

Pulse wave analysis (PWA)

Arterial stiffness will be measured using PWA. The procedure will be performed using a SphygmoCor device (version 7.0, Atcor Medical, Sydney, Australia) at the right radial artery.

Body composition

Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software provides a novel method for differentiating between visceral and subcutaneous abdominal fat.

Urine and blood samples, fasting

Standard blood analyses will be performed immediately after sampling. Blood and urine samples for specialized biomarkers will be stored at -80°C until batch analysis can be performed.

ENDPOINTS

Primary outcome measurement

The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow reserve MFR assessed by ^{82}Rb-PET

Key sub-study measurement

The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts

Secondary outcome measurements

The effect of empagliflozin compared with placebo on the following:

- Global left and right heart function
- Renal function by Cr51-EDTA plasma clearance
- Cardiac adipose tissue volume
- PCWP at peak exercise, corrected to body weight
- Plasma NT-proBNP concentrations
- Daily activity levels
- Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA

- Ambulatory systolic and diastolic blood pressures
- Quality of life assessed by the Minnesota Living with Heart Failure and the EuroQol EQ-5D-5L questionnaires

- Plasma beta-hydroxy butyrate levels
- Adipose tissue mRNA expression levels of TNF-α, COL1-A1 and monocyte chemoattractant protein 1 (MCP1)

STATISTICAL ANALYSIS

SAMPLE SIZE

Primary endpoint

The primary endpoint is change in MFR. In a previous study on a comparable population, the SD of measurements of MFR by ^{82}Rb PET was 0.8. Assuming a clinically relevant difference between the treatment and placebo groups after 6 months of 0.5 in MFR,[21] a sample size of 41:41 (empagliflozin: placebo) can be detected with 80% power and a two-sided significance level of 5%. In all, 92 patients will be randomized to allow for a 10% drop-out rate from the intention-to-treat (ITT) population.

Sub-study key endpoint

Based on recent experiments in a comparable study population,[22] a difference of 5 mmHg in PCWP during exercise was considered clinically significant. The SD of measurements of PCWP was 5 mmHg; thus, a sample size of 16:16 is required with a power of 80% and a two-sided significance level of 5% to detect a difference of 5 mmHg between groups. In total 38 patients will be included in the sub-study to allow for a 15% drop-out rate.

In the ITT population, none of the randomized participants will be excluded, and the patients will be analysed according to the randomization group. The per-protocol population will consist of all the patients who completed the study with a documented valid baseline and a final-week assessment of the primary objective without any major protocol violations. Analysis of the primary outcome parameter will focus on a change in global MBF from the baseline to week 13 between the treatment and control groups. The primary analysis will be based on the ITT population. The primary outcome measure will be analysed using ANCOVA with treatment as a factor and the baseline value as a covariate. The model will include the prespecified covariates age and gender. Missing data will be estimated using the maximum likelihood method. Normally distributed variables will be presented as mean ±SD, and non-parametric statistics or appropriate log transformation will be performed if an assumption of normality is not met. After log transformation, the variable will be further tested for normality distribution as indicated. A two-tailed *p* value of less than 0.05 will be considered statistically significant.

Comparisons between the treatment groups will be performed by an unpaired two sample *t*-test, Mann-Whitney test or χ2 - test as appropriate.

Data management

Source data will be recorded in the patient record or in case report forms (CRF). The requirements for entering source data directly into the CRF are that the data are obtained directly from the patient either by clinical assessment, interview or point-of-care systems with no printout function and that no more reliable forms of data capture are available. Medical history, height and weight are examples of such data.
A CRF will be constructed for data capture. Data will be stored in coded form for 5 years according to recommendations from the Danish Data Protection Agency; thereafter, data will be transferred to the Danish Data Archives.

Study medication

Name: Jardiance® (empagliflozin) or a visually identical matching placebo

Pharmaceutical Form: Tablet for oral use

Pharmaceutical Dosage: Jardiance® or a placebo will be introduced at a dose of 25 mg/day.
Intake of the tablet can be done at any time during the day; however, it is recommended that the time of intake be consistent from day to day.

Side effects: Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis, vulvovaginitis

Shipping and packing: All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

Randomization: Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned consecutively, and patients will retain these numbers following randomization.

Concomitant medication

Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving optimal T2D therapy, and during the trial, T2D and CV risk or disease will be managed according to the best available evidence.

ETHICS AND DISSEMINATION

The study, which will be conducted in accordance with the latest revision of the Helsinki Declaration, EU directive on GCP and ICH-GCP guidelines, has been approved by the Regional Scientific Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. The study is registered at clinicaltrials.gov and monitored by the GCP unit at Bispebjerg University Hospital. The results of the project will be submitted to international peer-reviewed journals regardless of their outcome, and the data will be made available to the public via EudraCT and www.clinicaltrials.gov. Furthermore, the results will be presented at conferences as abstracts and posters.

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Author Contributions:

CK, MS, FG, AK, PH, JF, BZ, SI and MJ conceived the study and participated in its design, planning and coordination. CK, MS, MW, PG and MJ are responsible for the inclusion and examination of patients at Herlev-Gentofte and Rigshospitalet University Hospital. AK and PH are responsible for the ^{82}Rb-PET measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.

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Competing interests:

PR has received consultancy and/or speaking fees (to his institution) from AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, MSD, Novo Nordisk and Sanofi Aventis and has received institutional research grants from AbbVie, AstraZeneca and Novo Nordisk.

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Table 1 Inclusion and exclusion criteria

Inclusion criteria

- T2D (WHO criteria), diagnosed at least 3 months before screening
- For patients on background therapy: stable dose of anti-diabetic therapy within 30 days prior to baseline
- Hba1c of ≥ 48 mmol/L and ≤ 86 mmol/L at screening for patients on background therapy or Hba1c of ≥ 48 mmol/L and ≤ 75 mmol/L at screening for drug-naïve patients
- Age ≥ 18 years
- BMI ≤ 45 kg/m
- Negative pregnancy test (fertile women). Fertile women must use safe contraceptives (spiral, hormonal contraceptives) for the duration of the study
- Able to understand the written patient information and to give informed consent
- High cardiovascular risk, defined as at least one of the following:
  - ACR ≥ 30 mg/g
  - NT-proBNP ≥ 70 pg/mL
  - Confirmed history of myocardial infarction > 2 months prior to baseline
  - Heart failure according to Framingham Heart Failure Criteria
  - Discharged from hospital with a documented diagnosis of UA ≤ 12 months prior to baseline
  - Evidence of coronary artery disease by CAG in one or more major coronary arteries OR at least one of the following: a positive noninvasive stress test, or a positive stress echocardiography showing regional systolic wall motion abnormalities, or a positive scintigraphic test showing stress-induced ischemia
  - History of ischemic or hemorrhagic stroke > 2 months prior to informed consent
  - Presence of peripheral artery disease such as: previous limb angioplasty, stenting or bypass surgery; or previous limb or foot amputation due to circulatory insufficiency; or angiographic evidence of significant (> 50%) peripheral artery stenosis in at least one limb; or evidence from a non-invasive measurement of significant (>50% or as reported as hemodynamically significant) peripheral artery stenosis in at least one limb; or ankle brachial index of < 0.9

Exclusion criteria

- Allergic to the study medication
- Treatment with SGLT-2 inhibitor within 1 month prior to baseline
- Impaired kidney function, eGFR ≤ 30 ml/min
- Severe liver insufficiency (Child-Pugh class C)
- ECG showing malign ventricular arrhythmia or prolonged QT-interval (>500ms)
- Untreated clinically significant heart valve disease
- Planned cardiac surgery or angioplasty within 3 months.
- Myocardial infarction (MI) ≤ 30 days prior to baseline
- Percutaneous coronary intervention (PCI) ≤ 4 weeks prior to baseline
- History of coronary artery bypass graft (CABG) ≤ 8 weeks prior to enrollment
- Prior history of heart transplantation
- Unstable angina, known severe left main coronary artery stenosis, severe heart failure, uncontrolled arrhythmias, symptomatic hypotension or severe hypertension (systolic blood pressure < 90 or > 180 mmHg, respectively), sick sinus syndrome or > first degree atrioventricular block in the absence of a functioning pacemaker

Table 2 Overview of study visits

Visit	V_0	V_1 Randomization	T_1	V_2	T_2	V_3
Time, weeks	-4±1	0±1	2±1	4±1	8±1	12±1
Inclusion/exclusion criteria	X	X				
Medical history	X					
Informed consent	X					
Blood samples, non-fasting	X			X		
Physical examination	X			X		
Adverse events		X	X	X	X	X
Endpoints, Main study						
^{82}Rb -PET		X			X	
Echocardiography		X			X	
Ambulatory BP		X			X	
Adipose tissue biopsy		X			X	
Oral glucose tolerance test		X			X	
Questionnaires		X			X	
HRV		X			X	
PWA		X			X	
^{51}Cr EDTA clearance		X			X	
Body composition (DEXA)		X			X	
Blood samples, fasting		X			X	
Urine samples, fasting		X			X	
Endpoints, substudy						
Hemodynamics		X			X	
Accelerometer		X			X	

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,11
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	See note 3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	See note 4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	14
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	See note 5
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return;	See note 6

		laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4-5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4-5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4-5, 11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4
Blinding (masking): emergency	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during	4-5

unblinding		the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	See note 7
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	See note 8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	5
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10

		whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	See note 9
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	See note 10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4,5,11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	See note 11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a (none)
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	See note 12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	10
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 13

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Author notes

1. n/a (not used)
2. 1 (The corresponding author is Sponsor-Investigator)
3. 1,11 (see above)
4. n/a (short, low risk trial)
5. n/a (no specific criteria)
6. n/a (no strategies)
7. n/a (no plans)
8. n/a (short, low risk study)
9. 2 (already approved)
10. n/a (no further modifications are expected)
11. 10 (public access)
12. n/a (standard guideline (Vancouver), no professional writers)
13. n/a (attached in seperate file)

BMJ Open

Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

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Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

Mikkel Jürgens[1], Morten Schou[2,3], Philip Hasbak[4], Andreas Kjær[4], Emil Wolsk[5], Bo Zerahn[6], Mikkel Wiberg[1], Niels Brandt[7], Peter Gæde[8], Peter Rossing[3,9], Jens Faber[1,3], Silvio E. Inzucchi[10], Finn Gustafsson[4,9], Caroline Kistorp[3,7]

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ABSTRACT

Introduction: A diagnosis of type 2 diabetes (T2D) more than doubles the risk of cardiovascular disease (CVD), with heart failure (HF) being one of the most common complications with a severe prognosis. The landmark EMPA-REG study demonstrated that treatment with the sodium glucose cotransporter-2 (SGLT-

2) inhibitor empagliflozin rapidly and significantly reduces CVD mortality and admission rates for HF. However, the mechanisms behind this reduction in clinical events are unknown.

The current study was designed to investigate the effects of the SGLT-2 inhibitor empagliflozin on myocardial perfusion and function in patients with T2D and high CVD risk.

Methods and analysis: In this investigator-initiated, randomized, double-blind controlled clinical trial, 92 patients with T2D and established CVD or high CVD risk, will be randomized to treatment with empagliflozin 25 mg or a matching placebo for 13 weeks. The primary outcome measure is change in myocardial flow reserve measured quantitatively by Rubidium-82 position emission tomography (^{82}Rb-PET). In a substudy, invasive hemodynamics at rest and during exercise will be measured at baseline and following the intervention, using right heart catheterization.

Ethics and dissemination: The study protocol (v7, 02/08/2018) has been approved by the Ethics Committee of the Capital Region, Danish Data Protection Board and the Danish Medicines Agency, and it will be monitored according to the Good Clinical Practice (GCP) regulations from the International Conference on Harmonization. The results be submitted to international peer-reviewed journals and be presented at conferences. The data will be made available to the public via EudraCT and www.clinicaltrials.gov.

Trial Registration: ClinicalTrials.gov Identifier: NCT03151343, EudraCT number: 2016-003743-10

ARTICLE SUMMARY

Strengths and limitations of this study:

- Double-blinded, randomized and placebo controlled.
- The use of advanced imaging techniques.
- Single-center
- No hard endpoints, all outcomes are based on surrogates

INTRODUCTION

Type 2 diabetes (T2D) is a significant risk factor for cardiovascular disease (CVD), including heart failure (HF), and improved glycaemic control is only modestly beneficial in reducing macrovascular disease.[1]

In 2008, prompted by concerns that the T2D drug rosiglitazone might increase the risk of myocardial infarction, the U.S. Food & Drug Administration published guidelines effectively mandating large cardiovascular outcome

trials for new T2D drugs. Several such trials have been completed or are ongoing. The trials have primarily examined the safety of dipeptidyl peptidase 4 (DPP-IV) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RA) and sodium–glucose cotransporter 2 (SGLT2) inhibitors.[2–8] The DPP-IV inhibitor trial SAVOR-TIMI indicated an increase in the secondary endpoint, hospitalization due to HF with the active comparator, while another DPP-IV trial, TECOS, as well as the newly published CARMELINA did not demonstrate an increased risk with the active drug. The GLP-1 RA trials LEADER and SUSTAIN although with a reduction in the primary MACE endpoint were neutral with regards to risk of HF. Most of the SGLT-2 trials have demonstrated CV benefits[9] via a reduction of the primary endpoint MACE. In the EMPA-REG outcome study, the SGLT-2 inhibitor empagliflozin reduced the risk of CV death by 38% and in contrast to the GLP-1 RA trials, admission due to HF by 35% in a high-risk T2D population.[6,10]

The novel evidence from the SGLT-2 inhibitor trials was surprising, because the treatments only had a modest effect on classical CV risk factors. Indeed, the mechanism behind their profound effect on CV death and risk of HF remains unknown, although several hypotheses have been proposed. An effect reducing the atherosclerotic burden is considered unlikely, because the risk of CV events was reduced within weeks of treatment[11]. Thus, the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is considered to play a significant role in T2D-related CVD[12] as well as in HF with preserved ejection fraction.[13] Furthermore, coronary vascular dysfunction, particularly decreased vasodilator capacity, is a significant predictor for CV death in patients with T2D.[14,15] Notably, T2D patients with preserved coronary vasodilation had the same low risk of CVD as subjects without diabetes[14]. Experimental studies have shown that SGLT-2 inhibitor treatment significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary arterial fibrosis and improves vasodilatation in diabetic rodents.[16,17] Thus, part of the underlying effect of empagliflozin in EMPA-REG could be driven by improvement in myocardial microcirculation.

A complementary hypothesis has been suggested to explain the early beneficial effects on HF hospitalization found in the EMPA-REG trial.[18] Inhibition of SGLT-2 is associated with increased diuresis, thus the sodium- and volume-reducing effect could favour cardiac loading conditions in patients with T2D with subclinical dysfunctions of the heart. The proposed effects on central hemodynamics, especially right and left heart filling pressures and cardiac output, can be measured directly by right heart catherization. Pulmonary capillary wedge pressure (PCWP), a measure of left heart filling pressure, has previously been shown to be associated with functional capacity among patients with HF.[19] Therefore, investigating the effect of SGLT-2 inhibitor on central hemodynamics during rest and exercise may contribute vital knowledge on the

mechanisms behind the markedly reduced risk of admission for HF demonstrated in EMPA-REG and DECLAIRE TIMI [6,8].

The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR) and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac function.[20] Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral glucose uptake[21] through a reduction in glucose toxicity. Moreover they induce a small increase in plasma ketone bodies, which may serve as a preferred fuel source for the myocardium.[22] Markers of inflammation have been associated with the risk of vascular events, independently of traditional risk factors and an effect on inflammation status should also be considered[23]. Therefore, further knowledge on the effects of SGLT-2 inhibitors on metabolic parameters, such as biomarkers of inflammation, adipose tissue function and the relationship with parameters on cardiac function in patients with T2D is warranted.

In summary, SGLT2 inhibitors have been shown to reduce CVD in high-risk T2D patients, with several explanatory hypotheses being suggested that remain to be tested in clinical trials.

Hypothesis

Treatment with empagliflozin for 13 weeks improves the myocardial flow reserve (MFR) in patients with T2D and high cardiovascular risk. The duration of 13 weeks was chosen, as the EMPA-REG study showed a clear effect on the primary outcomes at this timepoint.

Objectives

The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by ^{82}Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study, the effect of empagliflozin on key hemodynamic parameters will be measured during right heart catherization at rest and during exercise. Key secondary outcomes include changes in cardiac echocardiographic evaluations of systolic and diastolic functions, functional capacity by accelerometry, and changes in glucose metabolism, plasma ketone bodies and adipose tissue function.

METHODS AND ANALYSIS

Trial design

This is an investigator-initiated, randomized, placebo-controlled, double-blind trial. Participants will be recruited from the T2D and cardiology outpatient clinics at Herlev-Gentofte University Hospital, Steno Diabetes Center Copenhagen and Rigshospitalet, Denmark.

Study population

The study will include 92 patients with T2D who have either additional CV risk factors or pre-existing CVD. Detailed inclusion and exclusion criteria are listed in Table 1. Eligible patients will be invited for screening, and patients who meet all the criteria will be randomized to the double-blinded treatment. Participants will be considered part of the intention to treat (ITT) group after receiving the first dose of the study medication"

Trial Intervention

Participants will be randomized 1:1 to either empagliflozin 25 mg or matching placebo once daily for 13 weeks. A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicated a dose-response effect on metabolic outcomes[24]. The trial medication will be randomized in computer-generated 1:1 blocks of 10 by the study pharmacy (Glostrup Pharmacy, Copenhagen, Denmark). Participants will receive randomization numbers and the corresponding medication containers sequentially. In case of medical emergency, participants can be unblinded individually. The study medicine will be suspended if the participant becomes pregnant or withdraws consent. The investigators may suspend the medication for safety reasons.

Patient and public involvement

The study was designed without involvement from patients or the public. The patients will be offered access to the results of the study, when these become available.

Trial visits and procedures

Visits

A schematic overview of the trial visits is presented in Table 2 and as a flowchart in Figure 1. At the screening visit (V_0), informed consent will be obtained, and patients will be assessed for eligibility based on medical

history, including diabetes duration and comorbidities, physical examinations and blood samples. Ineligible participants will be counted as screen failures, and the reason for screen failure will be recorded.

Outcome-related procedures are performed at the baseline (V_1) and after 13 weeks of treatment (V_3). The trial medication will be dispensed when all the procedures pertaining to V_1 have been completed.

After 3 to 6 weeks of treatment, the participants will visit the trial site for blood tests and a physical examination (V_2). Participant status and compliance as well as adverse events will be assessed at V_2, V_3 and during two phone calls (T_1 and T_2).

^{82}Rb-PET

Myocardial perfusion and myocardial flow reserve (MFR) will be measured using cardiac ^{82}Rb-PET, which allows for flow quantification in absolute terms. To determine the MFR, measurements will be performed at rest and during adenosine-induced stress. A standard clinical protocol will be used. Participants will be scanned in the supine position using a Siemens Biograph mCT/PET 128-slice scanner (Siemens Medical Solutions, Knoxville, USA). Low-dose non-contrast CT will be acquired for attenuation correction as well as measurement of cardiac adipose tissue volume. Approximately 1100 MBq of ^{82}Rb will be obtained from a CardioGen-82 Sr-82/Rb-82 generator (Bracco Diagnostics, Inc., Princeton, New Jersey, USA) and intravenously infused with a constant flowrate of 50 mL/min. List-mode 3D data acquisition will begin with the tracer infusion and continue for 7 minutes. Static and ECG-gated images will be reconstructed with a 2.5-minute delay to allow ^{82}Rb to clear from the blood pool. Maximal hyperaemia will be induced with adenosine infused at 140 µg/kg/min for 6 minutes. After 2.5 minutes of adenosine infusion, intravenous ^{82}Rb infusion and list-mode acquisition will follow the same protocol as for rest. Myocardial blood perfusion quantification (in mL/min/g) will be performed using Cedars-Sinai QGS+QPS 2015.6 software (Cedars-Sinai Medical Center), which is based on a single-compartment model for Rb-82 tracer kinetics.

Hemodynamics sub-study

In a substudy, 38 participants randomly selected from the primary study population will perform a graded exercise test until maximal exertion using a supine ergometer with simultaneous invasive hemodynamic measurements. Using local anaesthesia, A Swan-Ganz catheter will be placed into the pulmonary artery via the right internal jugular vein. The following hemodynamic variables will be assessed: pulmonary capillary wedge pressure (PCWP), cardiac output (CO) using thermodilution, central venous pressure (CVP

and pulmonary artery pressure (PAP). Following measurements at rest, participants will be transferred to a supine bicycle ergometer, and the measurements will be repeated (leg-raise situation). The participant will be instructed to pedal at 60 rpm and the workload is incrementally increased at steps of 10watts. Measurements during exercise will be obtained at 25 watts and during peak exercise. Blood sampled from the pulmonary vein will be obtained at rest, 25 watts and at peak exercise for analyses of lactate, oxygen saturation and other blood gas variables.

Echocardiography

The following echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a') velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal speckle tracking) will be assessed via 2D echocardiography. The longitudinal tissue velocity of the LV will be assessed by averaging myocardial velocities and displacement of the mitral annular position in the septal, lateral, inferior, posterior and anterior wall of the LV. Diastolic function will be assessed according to EAE/ASE recommendations.

Cr-51 EDTA

The Cr-51 EDTA method will be used to measure glomerular filtration rate (GFR). A single intravenous administration of 3.7MBq of Cr-51 EDTA is given. A venous sample is drawn four hours after administration.

Accelerometry

A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the accelerometer continuously for 7 days, except for bathing and swimming.

Ambulatory blood pressure

24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every hour during the night.

Adipose tissue biopsy

Biopsies will be obtained from the abdominal subcutaneous tissue lateral to the umbilicus during a fasting state using the Bergstrom needle technique. Following dissection, the biopsies are washed and snap frozen in liquid nitrogen and stored at -80°C. Total mRNA will be extracted from adipose tissue with commercially available lipid tissue kits to measure the mRNA expression of biomarkers of interest. Protein expression will be analysed using Western blots.Quantification of inflammatory cells will be performed according to validated histological procedures. Fibrosis levels will be measured using the Sirius Red stain.

Oral Glucose Tolerance Test (OGTT)

The fasting participant will drink a solution of 75g of glucose. Blood will be drawn at time points 0, 30, 60 and 120 minutes, and measurements of plasma glucose, C-peptide and plasma insulin will be taken.

Questionnaires

Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.

Heart rate variability (HRV)

Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and while performing the Valsalva manoeuvre.

Pulse wave analysis (PWA)

Arterial stiffness will be measured using PWA. The procedure will be performed using a SphygmoCor device (version 7.0, Atcor Medical, Sydney, Australia) at the right radial artery.

Body composition

Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software provides a novel method for differentiating between visceral and subcutaneous abdominal fat.

Urine and blood samples, fasting

Blood will be drawn in the fasting state, and patients will provide a sample of first morning urine. Standard blood analyses will be performed immediately after sampling. Urine samples for measurement of albumin/creatinine ratio and blood samples for specialized biomarkers will be stored at -80°C until batch analysis can be performed. Blood analysis will include inflammatory biomarkers such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), cardiospecific biomarkers such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TNT), as well as markers of adipocyte function, including adiponectin and leptin.

ENDPOINTS

Primary outcome measurement

The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow reserve MFR assessed by ^{82}Rb-PET

Key sub-study measurement

The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts

Secondary outcome measurements

The effect of empagliflozin compared with placebo on the following:

- Global left and right heart function
- Renal function by Cr51-EDTA plasma clearance
- Cardiac adipose tissue volume
- PCWP at peak exercise, corrected to body weight
- Plasma levels of NT-proBNP , MR-proANP, MR-proADM, GAL-3, hsTNT, GDF-15, PIGF, sFlt-1, FFA, adiponectin, leptin, TNF-α, IL-6, MCP-1, MAC-1, COLL-A1 and FGF-21.
- Daily activity levels
- Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
- Ambulatory systolic and diastolic blood pressures

- Quality of life assessed by the Minnesota Living with Heart Failure and the EuroQol EQ-5D-5L questionnaires
- Plasma beta-hydroxy butyrate levels
- Urine albumin/creatinine ratio
- Adipose tissue fibrosis, mRNA and protein expression of TNF-α, adiponectin, IL-6, COL1-A1, MAC-1, FGF-21, and monocyte chemoattractant protein 1 (MCP1)

STATISTICAL ANALYSIS

SAMPLE SIZE

Primary endpoint The primary endpoint is change in MFR. In a previous study on a comparable population, the SD of measurements of MFR by ^{82}Rb PET was 0.8. Assuming a clinically relevant difference between the treatment and placebo groups after 6 months of 0.5 in MFR,[25] a sample size of 41:41 (empagliflozin: placebo) can be detected with 80% power and a two-sided significance level of 5%. In all, 92 patients will be randomized to allow for a 10% drop-out rate from the intention-to-treat (ITT) population.

Sub-study key endpoint

Based on recent experiments in a comparable study population,[26] a difference of 5 mmHg in PCWP during exercise was considered clinically significant. The SD of measurements of PCWP was 5 mmHg; thus, a sample size of 16:16 is required with a power of 80% and a two-sided significance level of 5% to detect a difference of 5 mmHg between groups. In total 38 patients will be included in the sub-study to allow for a 15% drop-out rate.

In the ITT population, none of the randomized participants will be excluded, and the patients will be analysed according to the randomization group. The per-protocol population will consist of all the patients who completed the study with a documented valid baseline and a final-week assessment of the primary objective without any major protocol violations. Analysis of the primary outcome parameter will focus on a change in global MBF from the baseline to week 13 between the treatment and control groups. The primary analysis will be based on the ITT population. The primary outcome measure will be analysed using ANCOVA with treatment as a factor and the baseline value as a covariate. The model will include the prespecified covariates age and gender. Missing data will be estimated using the maximum likelihood method. Normally distributed variables will be presented as mean ±SD, and non-parametric statistics or appropriate log transformation will be

performed if an assumption of normality is not met. After log transformation, the variable will be further tested for normality distribution as indicated. A two-tailed *p* value of less than 0.05 will be considered statistically significant.

Comparisons between the treatment groups will be performed by an unpaired two sample *t*-test, Mann-Whitney test or χ2 - test as appropriate.

Data management

Source data will be recorded in the patient record or in case report forms (CRF). The requirements for entering source data directly into the CRF are that the data are obtained directly from the patient either by clinical assessment, interview or point-of-care systems with no printout function and that no more reliable forms of data capture are available. Medical history, height and weight are examples of such data.

A CRF will be constructed for data capture. Data will be stored in coded form for 5 years according to recommendations from the Danish Data Protection Agency; thereafter, data will be transferred to the Danish Data Archives.

Study medication

Name: Jardiance® (empagliflozin) or a visually identical matching placebo

Pharmaceutical Form: Tablet for oral use

Pharmacological Dosage: Jardiance® or a placebo will be introduced at a dose of 25 mg/day.

Intake of the tablet can be done at any time during the day; however, it is recommended that the time of intake be consistent from day to day.

Side effects: Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis, vulvovaginitis

Shipping and packing: All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

Randomization: Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned consecutively, and patients will retain these numbers following randomization.

Concomitant medication

Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving optimal T2D therapy, and during the trial, T2D and CV risk or disease will be managed according to the best available evidence.

ETHICS AND DISSEMINATION

The study, which will be conducted in accordance with the latest revision of the Helsinki Declaration, EU directive on GCP and ICH-GCP guidelines, has been approved by the Regional Scientific Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. The study is registered at clinicaltrials.gov and monitored by the GCP unit at Bispebjerg University Hospital. The results of the project will be submitted to international peer-reviewed journals regardless of their outcome, and the data will be made available to the public via EudraCT and www.clinicaltrials.gov. Furthermore, the results will be presented at conferences as abstracts and posters.

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Author Contributions:

CK, MS, FG, AK, PH, JF, BZ, SI and MJ conceived the study and participated in its design, planning and coordination. CK, MS, MW, PG, PR, NB and MJ are responsible for the inclusion and examination of patients at Herlev-Gentofte and Rigshospitalet University Hospital. AK and PH are responsible for the ^{82}Rb-PET measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.

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ClinicalTrials.gov Identifier: NCT03151343, EudraCT number: 2016-003743-10

Competing interests:

PR has received consultancy and/or speaking fees (to his institution) from AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, MSD, Novo Nordisk and Sanofi Aventis and has received institutional research grants from AbbVie, AstraZeneca and Novo Nordisk.

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Table 1 Inclusion and exclusion criteria

Inclusion criteria

- T2D (WHO criteria), diagnosed at least 3 months before screening
- For patients on background therapy: No change in anti-diabetic therapy within 30 days prior to baseline
- Hba1c of ≥ 48 mmol/L and ≤ 86 mmol/L at screening for patients on background therapy or Hba1c of ≥ 48 mmol/L and ≤ 75 mmol/L at screening for drug-naïve patients
- Age ≥ 18 years
- BMI ≤ 45 kg/m
- Negative pregnancy test (fertile women). Fertile women must use safe contraceptives (spiral, hormonal contraceptives) for the duration of the study
- Able to understand the written patient information and to give informed consent
- High cardiovascular risk, defined as at least one of the following:
  - ACR ≥ 30 mg/g
  - NT-proBNP ≥ 70 pg/mL
  - Confirmed history of myocardial infarction > 2 months prior to baseline
  - Heart failure according to Framingham Heart Failure Criteria
  - Discharged from hospital with a documented diagnosis of UA ≤ 12 months prior to baseline
  - Evidence of coronary artery disease by CAG in one or more major coronary arteries OR at least one of the following: a positive noninvasive stress test, or a positive stress echocardiography showing regional systolic wall motion abnormalities, or a positive scintigraphic test showing stress-induced ischemia
  - History of ischemic or hemorrhagic stroke > 2 months prior to informed consent
  - Presence of peripheral artery disease such as: previous limb angioplasty, stenting or bypass surgery; or previous limb or foot amputation due to circulatory insufficiency; or angiographic evidence of significant (> 50%) peripheral artery stenosis in at least one limb; or evidence from a non-invasive measurement of significant (>50% or as reported as hemodynamically significant) peripheral artery stenosis in at least one limb; or ankle brachial index of < 0.9

Exclusion criteria

- Allergic to the study medication
- Treatment with SGLT-2 inhibitor within 1 month prior to baseline
- Impaired kidney function, eGFR ≤ 30 ml/min
- Severe liver insufficiency (Child-Pugh class C)
- ECG showing malign ventricular arrhythmia or prolonged QT-interval (>500ms)
- Untreated clinically significant heart valve disease
- Planned cardiac surgery or angioplasty within 3 months.
- Myocardial infarction (MI) ≤ 30 days prior to baseline
- Percutaneous coronary intervention (PCI) ≤ 4 weeks prior to baseline
- History of coronary artery bypass graft (CABG) ≤ 8 weeks prior to enrollment
- Prior history of heart transplantation
- Unstable angina, known severe left main coronary artery stenosis, severe heart failure, uncontrolled arrhythmias, symptomatic hypotension or severe hypertension (systolic blood pressure < 90 or > 180 mmHg, respectively), sick sinus syndrome or > first degree atrioventricular block in the absence of a functioning pacemaker
- Requirement of emergent cardiac medical intervention or catheterization
- Treatment with theophylline, or medications containing theophyline
- History of known or suspected bronchoconstrictive or bronchospastic lung disease (e.g., asthma)
- Not using safe contraception
- Pregnancy or desire hereof or breastfeeding.

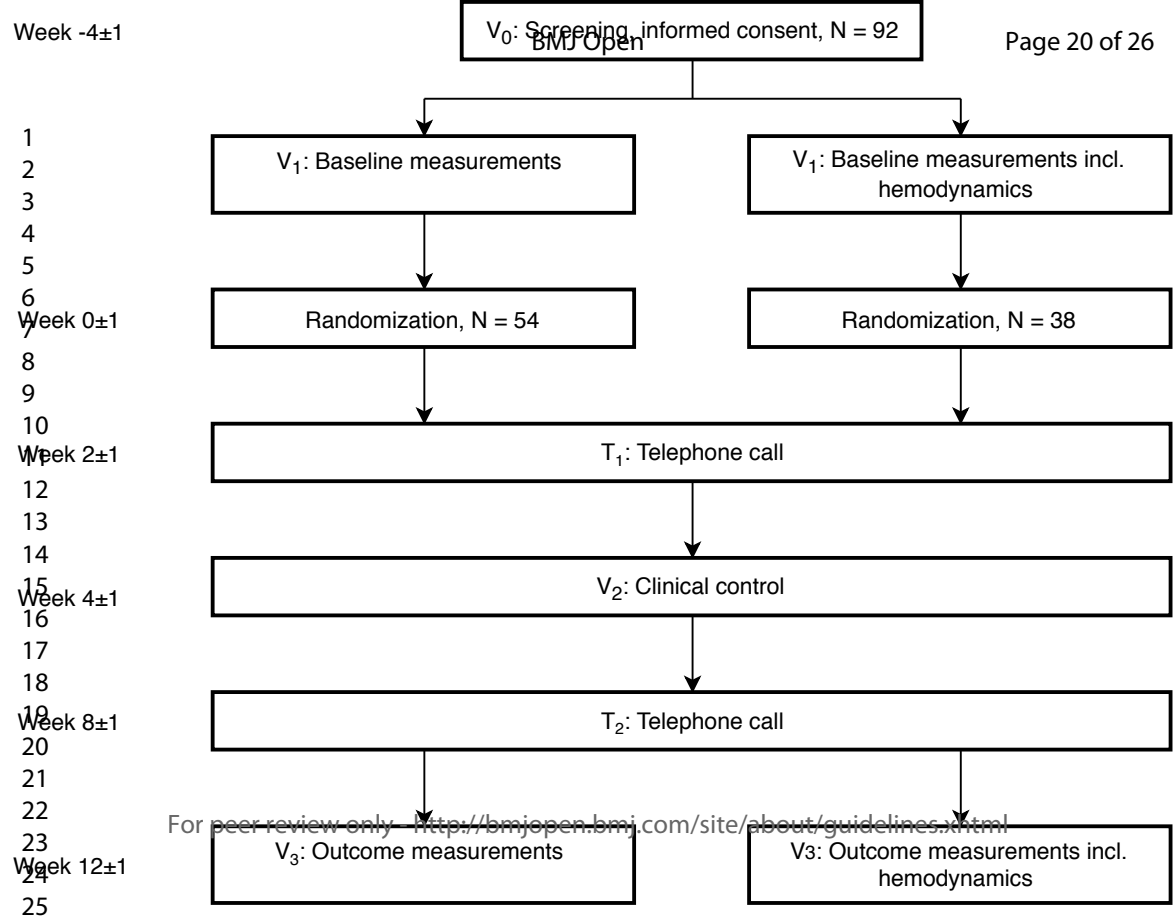
ACR, albumin creatinine ratio; BMI, body mass index; CAG, coronary angiography; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; UA, unstable angina

Table 2 Overview of study visits

Visit	V_0	V_1 Randomization	T_1	V_2	T_2	V_3
Time, weeks	-4±1	0±1	2±1	4±1	8±1	12±1
Inclusion/exclusion criteria	X	X				
Medical history	X					
Informed consent	X					
Blood samples, non-fasting	X			X		
Physical examination	X			X		
Adverse events		X	X	X	X	X
Endpoints, Main study						
^{82}Rb -PET (primary endpoint)		X				X
Echocardiography		X				X
Ambulatory BP		X				X
Adipose tissue biopsy		X				X
Oral glucose tolerance test		X				X
Questionnaires		X				X
HRV		X				X
PWA		X				X
^{51}Cr EDTA clearance		X				X
Body composition (DEXA)		X				X
Blood samples, fasting		X				X
Urine samples, fasting		X				X
Endpoints, substudy						
Hemodynamics (key secondary endpoint)		X				X
Accelerometer		X				X

Figure 1

Time-line of the study visits



Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,11
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	See note 3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	See note 4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	14
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	See note 5
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return;	See note 6

		laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4-5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4-5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4-5, 11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4
Blinding (masking): emergency	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during	4-5

unblinding		the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	See note 7
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	See note 8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	5
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10

		whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	See note 9
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	See note 10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4,5,11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	See note 11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a (none)
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	See note 12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	10
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 13

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Author notes

1. n/a (not used)
2. 1 (The corresponding author is Sponsor-Investigator)
3. 1,11 (see above)
4. n/a (short, low risk trial)
5. n/a (no specific criteria)
6. n/a (no strategies)
7. n/a (no plans)
8. n/a (short, low risk study)
9. 2 (already approved)
10. n/a (no further modifications are expected)
11. 10 (public access)
12. n/a (standard guideline (Vancouver), no professional writers)
13. n/a (attached in seperate file)

BMJ Open

Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-029098.R2
Article Type:	Protocol
Date Submitted by the Author:	11-Sep-2019
Complete List of Authors:	Jürgens, Mikkel; Herlev Hospital, Schou, Morten; Herlev Hospital, Cardiology Hasbak, Philip; Copenhagen University Hospital, Rigshospitalet, Clinical Physiology, Nuclear Medicine & PET 4011, Cluster of Molecular Imaging kjær, andreas; Copenhagen University Hospital, Rigshospitalet, Clinical Physiology, Nuclear Medicine & PET 4011, Cluster of Molecular Imaging Wolsk, Emil; Rigshospitalet, Department of Cardiology - The Heart Centre Zerahn, Bo; Herlev and Gentofte Hospital, University of Copenhagen, Department of Clinical Physiology and Nuclear Medicine Wiberg, Mikkel; Herlev Hospital, Medicine Brandt, Niels; Herlev and Gentofte Hospital, University of Copenhagen, Deparment of medicine Gæde, Peter; Slagelse Sygehus Rossing, Peter; Steno Diabetes Center AS Faber, J; Herlev Hospital, Medicine Inzucchi, Silvio; Yale Shool of Medicine, Section of Endocrinology Gustafsson, Finn; Rigshospitalet, Department of Cardiology - The Heart Centre Kistorp, Caroline; Rigshospitalet, Department of Endocrinology
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Diabetic nephropathy & vascular disease < DIABETES & ENDOCRINOLOGY, CARDIOLOGY, Cardiovascular imaging < RADIOLOGY & IMAGING

Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

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ABSTRACT

Introduction: A diagnosis of type 2 diabetes (T2D) more than doubles the risk of cardiovascular disease (CVD), with heart failure (HF) being one of the most common complications with a severe prognosis. The landmark EMPA-REG study demonstrated that treatment with the sodium glucose cotransporter-2 (SGLT-

2) inhibitor empagliflozin rapidly and significantly reduces CVD mortality and admission rates for HF. However, the mechanisms behind this reduction in clinical events are unknown.

The current study was designed to investigate the effects of the SGLT-2 inhibitor empagliflozin on myocardial perfusion and function in patients with T2D and high CVD risk.

Methods and analysis: In this investigator-initiated, randomized, double-blind controlled clinical trial, 92 patients with T2D and established CVD or high CVD risk, will be randomized to treatment with empagliflozin 25 mg or a matching placebo for 13 weeks. The primary outcome measure is change in myocardial flow reserve measured quantitatively by Rubidium-82 position emission tomography (^{82}Rb-PET). In a substudy, invasive hemodynamics at rest and during exercise will be measured at baseline and following the intervention, using right heart catheterization.

Ethics and dissemination: The study protocol (v7, 02/08/2018) has been approved by the Ethics Committee of the Capital Region, Danish Data Protection Board and the Danish Medicines Agency, and it will be monitored according to the Good Clinical Practice (GCP) regulations from the International Conference on Harmonization. The results be submitted to international peer-reviewed journals and be presented at conferences. The data will be made available to the public via EudraCT and www.clinicaltrials.gov.

Trial Registration: ClinicalTrials.gov Identifier: NCT03151343, EudraCT number: 2016-003743-10

ARTICLE SUMMARY

Strengths and limitations of this study:

- Double-blinded, randomized and placebo controlled.
- The use of advanced imaging techniques.
- Single-center
- No hard endpoints, all outcomes are based on surrogates

INTRODUCTION

Type 2 diabetes (T2D) is a significant risk factor for cardiovascular disease (CVD), including heart failure (HF), and improved glycaemic control is only modestly beneficial in reducing macrovascular disease.[1]

In 2008, prompted by concerns that the T2D drug rosiglitazone might increase the risk of myocardial infarction, the U.S. Food & Drug Administration published guidelines effectively mandating large cardiovascular outcome

trials for new T2D drugs. Several such trials have been completed or are ongoing. The trials have primarily examined the safety of dipeptidyl peptidase 4 (DPP-IV) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RA) and sodium–glucose cotransporter 2 (SGLT2) inhibitors.[2–8] The DPP-IV inhibitor trial SAVOR-TIMI indicated an increase in the secondary endpoint, hospitalization due to HF with the active comparator, while another DPP-IV trial, TECOS, as well as the newly published CARMELINA did not demonstrate an increased risk with the active drug. The GLP-1 RA trials LEADER and SUSTAIN although with a reduction in the primary MACE endpoint were neutral with regards to risk of HF. Most of the SGLT-2 trials have demonstrated CV benefits[9] via a reduction of the primary endpoint MACE. In the EMPA-REG outcome study, the SGLT-2 inhibitor empagliflozin reduced the risk of CV death by 38% and in contrast to the GLP-1 RA trials, admission due to HF by 35% in a high-risk T2D population.[6,10]

The novel evidence from the SGLT-2 inhibitor trials was surprising, because the treatments only had a modest effect on classical CV risk factors. Indeed, the mechanism behind their profound effect on CV death and risk of HF remains unknown, although several hypotheses have been proposed. An effect reducing the atherosclerotic burden is considered unlikely, because the risk of CV events was reduced within weeks of treatment[11]. Thus, the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is considered to play a significant role in T2D-related CVD[12] as well as in HF with preserved ejection fraction.[13] Furthermore, coronary vascular dysfunction, particularly decreased vasodilator capacity, is a significant predictor for CV death in patients with T2D.[14,15] Notably, T2D patients with preserved coronary vasodilation had the same low risk of CVD as subjects without diabetes[14]. Experimental studies have shown that SGLT-2 inhibitor treatment significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary arterial fibrosis and improves vasodilatation in diabetic rodents.[16,17] Thus, part of the underlying effect of empagliflozin in EMPA-REG could be driven by improvement in myocardial microcirculation.

A complementary hypothesis has been suggested to explain the early beneficial effects on HF hospitalization found in the EMPA-REG trial.[18] Inhibition of SGLT-2 is associated with increased diuresis, thus the sodium- and volume-reducing effect could favour cardiac loading conditions in patients with T2D with subclinical dysfunctions of the heart. The proposed effects on central hemodynamics, especially right and left heart filling pressures and cardiac output, can be measured directly by right heart catherization. Pulmonary capillary wedge pressure (PCWP), a measure of left heart filling pressure, has previously been shown to be associated with functional capacity among patients with HF.[19] Therefore, investigating the effect of SGLT-2 inhibitor on central hemodynamics during rest and exercise may contribute vital knowledge on the

mechanisms behind the markedly reduced risk of admission for HF demonstrated in EMPA-REG and DECLAIRE TIMI [6,8].

The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR) and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac function.[20] Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral glucose uptake[21] through a reduction in glucose toxicity. SGLT-2 inhibitors have been shown to increase the glucagon-insulin ratio, presumably due to the decrease in glucose stimulus.[22] Moreover they induce a small increase in plasma ketone bodies, which some have proposed to be a preferred fuel source for the myocardium.[23] However, others have shown that SGLT-2 inhibitors increase ATP production in mouse model hearts, without increasing ketone oxidation.[24] Markers of inflammation have been associated with the risk of vascular events, independently of traditional risk factors and an effect on markers of inflammation status such as IL-6 and CRP should also be considered.[25] Therefore, further knowledge on the effects of SGLT-2 inhibitors on metabolic parameters, such as biomarkers of inflammation, adipose tissue function and the relationship with parameters on cardiac function in patients with T2D is warranted.

In summary, SGLT2 inhibitors have been shown to reduce CVD in high-risk T2D patients, with several explanatory hypotheses being suggested that remain to be tested in clinical trials.

Hypothesis

Treatment with empagliflozin for 13 weeks improves the myocardial flow reserve (MFR) in patients with T2D and high cardiovascular risk. The duration of 13 weeks was chosen, as the EMPA-REG study showed a clear effect on the primary outcomes at this timepoint.

Objectives

The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by ^{82}Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study, the effect of empagliflozin on key hemodynamic parameters will be measured during right heart catherization at rest and during exercise. Key secondary outcomes include changes in cardiac echocardiographic evaluations of systolic and diastolic functions, functional capacity by accelerometry, and changes in glucose metabolism, plasma ketone bodies and adipose tissue function.

METHODS AND ANALYSIS

Trial design

This is an investigator-initiated, randomized, placebo-controlled, double-blind trial. Participants will be recruited from the T2D and cardiology outpatient clinics at Herlev-Gentofte University Hospital, Steno Diabetes Center Copenhagen and Rigshospitalet, Denmark.

Study population

The study will include 92 patients who have had the T2D diagnosis for at least 3 months, and with no upper limit to the duration of diabetes, who have either additional CV risk factors or pre-existing CVD. Detailed inclusion and exclusion criteria are listed in Table 1. Eligible patients will be invited for screening, and patients who meet all the criteria will be randomized to the double-blinded treatment. Participants will be considered part of the intention to treat (ITT) group after receiving the first dose of the study medication"

Trial Intervention

Participants will be randomized 1:1 to either empagliflozin 25 mg or matching placebo once daily for 13 weeks. A cross-over design was considered but not implemented, due to the expected high availability of eligible participants. A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicated a dose-response effect on metabolic outcomes such as urinary glucose excretion, fasting plasma glucose and mean daily plasma glucose, with higher drug doses resulting in lower glucose levels and increased glucose excretion.[26]. In accordance with the EMPA-REG study, no dose-escalation will be performed. The trial medication will be randomized in computer-generated 1:1 blocks of 10 by the study pharmacy (Glostrup Pharmacy, Copenhagen, Denmark). Participants will receive randomization numbers and the corresponding medication containers sequentially. In case of medical emergency, participants can be unblinded individually. The study medicine will be suspended if the participant becomes pregnant or withdraws consent. The investigators may suspend the medication for safety reasons.

Patient and public involvement

The study was designed without involvement from patients or the public. The patients will be offered access to the results of the study, when these become available.

Trial visits and procedures

Visits

A schematic overview of the trial visits is presented in Table 2 and as a flowchart in Figure 1. At the screening visit (V_0), informed consent will be obtained, and patients will be assessed for eligibility based on medical history, including diabetes duration and comorbidities, physical examinations and blood samples. Ineligible participants will be counted as screen failures, and the reason for screen failure will be recorded.

Outcome-related procedures are performed at the baseline (V_1) and after 13 weeks of treatment (V_3). The trial medication will be dispensed when all the procedures pertaining to V_1 have been completed.

After 3 to 6 weeks of treatment, the participants will visit the trial site for blood tests and a physical examination (V_2). Participant status and compliance as well as adverse events will be assessed at V_2, V_3 and during two phone calls (T_1 and T_2).

^{82}Rb-PET

Myocardial perfusion and myocardial flow reserve (MFR) will be measured using cardiac ^{82}Rb-PET, which allows for flow quantification in absolute terms. To determine the MFR, measurements will be performed at rest and during adenosine-induced stress. A standard clinical protocol will be used. Participants will be scanned in the supine position using a Siemens Biograph mCT/PET 128-slice scanner (Siemens Medical Solutions, Knoxville, USA). Low-dose non-contrast CT will be acquired for attenuation correction as well as measurement of cardiac adipose tissue volume. Approximately 1100 MBq of ^{82}Rb will be obtained from a CardioGen-82 Sr-82/Rb-82 generator (Bracco Diagnostics, Inc., Princeton, New Jersey, USA) and intravenously infused with a constant flowrate of 50 mL/min. List-mode 3D data acquisition will begin with the tracer infusion and continue for 7 minutes. Static and ECG-gated images will be reconstructed with a 2.5-minute delay to allow ^{82}Rb to clear from the blood pool. Maximal hyperaemia will be induced with adenosine infused at 140 µg/kg/min for 6 minutes. After 2.5 minutes of adenosine infusion, intravenous ^{82}Rb infusion and list-mode acquisition will follow the same protocol as for rest. Myocardial blood perfusion quantification (in mL/min/g) will be performed using Cedars-Sinai QGS+QPS 2015.6 software (Cedars-Sinai Medical Center), which is based on a single-compartment model for Rb-82 tracer kinetics.

Hemodynamics sub-study

In a substudy, 38 participants randomly selected from the primary study population will perform a graded exercise test until maximal exertion using a supine ergometer with simultaneous invasive hemodynamic measurements. Using local anaesthesia, A Swan-Ganz catheter will be placed into the pulmonary artery via the right internal jugular vein. The following hemodynamic variables will be assessed: pulmonary capillary wedge pressure (PCWP), cardiac output (CO) using thermodilution, central venous pressure (CVP and pulmonary artery pressure (PAP). Following measurements at rest, participants will be transferred to a supine bicycle ergometer, and the measurements will be repeated (leg-raise situation). The participant will be instructed to pedal at 60 rpm and the workload is incrementally increased at steps of 10watts. Measurements during exercise will be obtained at 25 watts and during peak exercise. Blood sampled from the pulmonary vein will be obtained at rest, 25 watts and at peak exercise for analyses of lactate, oxygen saturation and other blood gas variables.

Echocardiography

The following echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a') velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal speckle tracking) will be assessed via 2D echocardiography. The longitudinal tissue velocity of the LV will be assessed by averaging myocardial velocities and displacement of the mitral annular position in the septal, lateral, inferior, posterior and anterior wall of the LV. Diastolic function will be assessed according to EAE/ASE recommendations.

Cr-51 EDTA

The Cr-51 EDTA method will be used to measure glomerular filtration rate (GFR). A single intravenous administration of 3.7MBq of Cr-51 EDTA is given. A venous sample is drawn four hours after administration.

Accelerometry

A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the accelerometer continuously for 7 days, except for bathing and swimming.

Ambulatory blood pressure

24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every hour during the night.

Adipose tissue biopsy

Biopsies will be obtained from the abdominal subcutaneous tissue lateral to the umbilicus during a fasting state using the Bergstrom needle technique. Following dissection, the biopsies are washed and snap frozen in liquid nitrogen and stored at -80°C. Total mRNA will be extracted from adipose tissue with commercially available lipid tissue kits to measure the mRNA expression of biomarkers of interest. Protein expression will be analysed using Western blots. Quantification of inflammatory cells will be performed according to validated histological procedures. Fibrosis levels will be measured using the Sirius Red stain.

Oral Glucose Tolerance Test (OGTT)

The fasting participant will drink a solution of 75g of glucose. Blood will be drawn at time points 0, 30, 60 and 120 minutes, and measurements of plasma glucose, C-peptide and plasma insulin will be taken. Frozen aliquots for measurement of glucagon will be kept for a later sub-study.

Questionnaires

Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.

Heart rate variability (HRV)

Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and while performing the Valsalva manoeuvre.

Pulse wave analysis (PWA)

Arterial stiffness will be measured using PWA. The procedure will be performed using a SphygmoCor device (version 7.0, Atcor Medical, Sydney, Australia) at the right radial artery.

Body composition

Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software provides a novel method for differentiating between visceral and subcutaneous abdominal fat.

Urine and blood samples, fasting

Blood will be drawn in the fasting state, and patients will provide a sample of first morning urine. Standard blood analyses will be performed immediately after sampling. Urine samples for measurement of albumin/creatinine ratio and blood samples for specialized biomarkers will be stored at -80°C until batch analysis can be performed. Blood analysis will include inflammatory biomarkers such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), cardiospecific biomarkers such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TNT), as well as markers of adipocyte function, including adiponectin and leptin.

ENDPOINTS

Primary outcome measurement

The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow reserve MFR assessed by ^{82}Rb-PET

Key sub-study measurement

The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts

Secondary outcome measurements

The effect of empagliflozin compared with placebo on the following:

- Global left and right heart function
- Renal function by Cr51-EDTA plasma clearance
- Cardiac adipose tissue volume
- PCWP at peak exercise, corrected to body weight

- Plasma levels of NT-proBNP , MR-proANP, MR-proADM, GAL-3, hsTNT, GDF-15, PIGF, sFlt-1, FFA, adiponectin, leptin, TNF-α, IL-6, MCP-1, MAC-1, COLL-A1, endothelin-1 and FGF-21.
- Daily activity levels
- Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
- Ambulatory systolic and diastolic blood pressures
- Quality of life assessed by the Minnesota Living with Heart Failure and the EuroQol EQ-5D-5L questionnaires
- Plasma beta-hydroxy butyrate levels
- Urine albumin/creatinine ratio
- Adipose tissue fibrosis, mRNA and protein expression of TNF-α, adiponectin, IL-6, COL1-A1, MAC-1, FGF-21, and monocyte chemoattractant protein 1 (MCP1)

STATISTICAL ANALYSIS

SAMPLE SIZE

Primary endpoint The primary endpoint is change in MFR. In a previous study on a comparable population, the SD of measurements of MFR by ^{82}Rb PET was 0.8. Assuming a clinically relevant difference between the treatment and placebo groups after 6 months of 0.5 in MFR,[27] a sample size of 41:41 (empagliflozin: placebo) can be detected with 80% power and a two-sided significance level of 5%. In all, 92 patients will be randomized to allow for a 10% drop-out rate from the intention-to-treat (ITT) population.

Sub-study key endpoint

Based on recent experiments in a comparable study population,[28] a difference of 5 mmHg in PCWP during exercise was considered clinically significant. The SD of measurements of PCWP was 5 mmHg; thus, a sample size of 16:16 is required with a power of 80% and a two-sided significance level of 5% to detect a difference of 5 mmHg between groups. In total 38 patients will be included in the sub-study to allow for a 15% drop-out rate.

In the ITT population, none of the randomized participants will be excluded, and the patients will be analysed according to the randomization group. The per-protocol population will consist of all the patients who completed the study with a documented valid baseline and a final-week assessment of the primary objective without any major protocol violations. Analysis of the primary outcome parameter will focus on a change in

global MBF from the baseline to week 13 between the treatment and control groups. The primary analysis will be based on the ITT population. The primary outcome measure will be analysed using ANCOVA with treatment as a factor and the baseline value as a covariate. The model will include the prespecified covariates age and gender. Missing data will be estimated using the maximum likelihood method. Normally distributed variables will be presented as mean ±SD, and non-parametric statistics or appropriate log transformation will be performed if an assumption of normality is not met. After log transformation, the variable will be further tested for normality distribution as indicated. A two-tailed *p* value of less than 0.05 will be considered statistically significant.

Comparisons between the treatment groups will be performed by an unpaired two sample *t*-test, Mann-Whitney test or χ2 - test as appropriate.

Data management

Source data will be recorded in the patient record or in case report forms (CRF). The requirements for entering source data directly into the CRF are that the data are obtained directly from the patient either by clinical assessment, interview or point-of-care systems with no printout function and that no more reliable forms of data capture are available. Medical history, height and weight are examples of such data.

A CRF will be constructed for data capture. Data will be stored in coded form for 5 years according to recommendations from the Danish Data Protection Agency; thereafter, data will be transferred to the Danish Data Archives.

Study medication

Name: Jardiance® (empagliflozin) or a visually identical matching placebo

Pharmaceutical Form: Tablet for oral use

Pharmacological Dosage: Jardiance® or a placebo will be introduced at a dose of 25 mg/day.

Intake of the tablet can be done at any time during the day; however, it is recommended that the time of intake be consistent from day to day.

Side effects: Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis, vulvovaginitis

Shipping and packing: All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

Randomization: Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned consecutively, and patients will retain these numbers following randomization.

Concomitant medication

Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving optimal T2D therapy, and during the trial, T2D and CV risk or disease will be managed according to the best available evidence.

ETHICS AND DISSEMINATION

The study, which will be conducted in accordance with the latest revision of the Helsinki Declaration, EU directive on GCP and ICH-GCP guidelines, has been approved by the Regional Scientific Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. The study is registered at clinicaltrials.gov and monitored by the GCP unit at Bispebjerg University Hospital. The results of the project will be submitted to international peer-reviewed journals regardless of their outcome, and the data will be made available to the public via EudraCT and www.clinicaltrials.gov. Furthermore, the results will be presented at conferences as abstracts and posters.

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Author Contributions:

CK, MS, FG, AK, PH, JF, BZ, SI and MJ conceived the study and participated in its design, planning and coordination. CK, MS, MW, PG, PR, NB and MJ are responsible for the inclusion and examination of patients at Herlev-Gentofte and Rigshospitalet University Hospital. AK and PH are responsible for the ^{82}Rb-PET measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.

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Competing interests:

PR has received consultancy and/or speaking fees (to his institution) from AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, MSD, Novo Nordisk and Sanofi Aventis and has received institutional research grants from AbbVie, AstraZeneca and Novo Nordisk.

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Table 1 Inclusion and exclusion criteria

Inclusion criteria

- T2D (WHO criteria), diagnosed at least 3 months before screening, and with no upper limit to duration
- For patients on background therapy: No change in anti-diabetic therapy within 30 days prior to baseline
- Hba1c of ≥ 48 mmol/L and ≤ 86 mmol/L at screening for patients on background therapy or Hba1c of ≥ 48 mmol/L and ≤ 75 mmol/L at screening for drug-naïve patients
- Age ≥ 18 years
- BMI ≤ 45 kg/m
- Negative pregnancy test (fertile women). Fertile women must use safe contraceptives (spiral, hormonal contraceptives) for the duration of the study
- Able to understand the written patient information and to give informed consent
- High cardiovascular risk, defined as at least one of the following:
  - ACR ≥ 30 mg/g
  - NT-proBNP ≥ 70 pg/mL
  - Confirmed history of myocardial infarction > 2 months prior to baseline
  - Heart failure according to Framingham Heart Failure Criteria
  - Discharged from hospital with a documented diagnosis of UA ≤ 12 months prior to baseline
  - Evidence of coronary artery disease by CAG in one or more major coronary arteries OR at least one of the following: a positive noninvasive stress test, or a positive stress echocardiography showing regional systolic wall motion abnormalities, or a positive scintigraphic test showing stress-induced ischemia
  - History of ischemic or hemorrhagic stroke > 2 months prior to informed consent
  - Presence of peripheral artery disease such as: previous limb angioplasty, stenting or bypass surgery; or previous limb or foot amputation due to circulatory insufficiency; or angiographic evidence of significant (> 50%) peripheral artery stenosis in at least one limb; or evidence from a non-invasive measurement of significant (>50% or as reported as hemodynamically significant) peripheral artery stenosis in at least one limb; or ankle brachial index of < 0.9

Exclusion criteria

- Allergic to the study medication
- Treatment with SGLT-2 inhibitor within 1 month prior to baseline
- Impaired kidney function, eGFR ≤ 30 ml/min
- Severe liver insufficiency (Child-Pugh class C)
- ECG showing malign ventricular arrhythmia or prolonged QT-interval (>500ms)
- Untreated clinically significant heart valve disease
- Planned cardiac surgery or angioplasty within 3 months.
- Myocardial infarction (MI) ≤ 30 days prior to baseline
- Percutaneous coronary intervention (PCI) ≤ 4 weeks prior to baseline
- History of coronary artery bypass graft (CABG) ≤ 8 weeks prior to enrollment
- Prior history of heart transplantation
- Unstable angina, known severe left main coronary artery stenosis, severe heart failure, uncontrolled arrhythmias, symptomatic hypotension or severe hypertension (systolic blood pressure < 90 or > 180 mmHg, respectively), sick sinus syndrome or > first degree atrioventricular block in the absence of a functioning pacemaker
- Requirement of emergent cardiac medical intervention or catheterization
- Treatment with theophylline, or medications containing theophyline
- History of known or suspected bronchoconstrictive or bronchospastic lung disease (e.g., asthma)
- Not using safe contraception
- Pregnancy or desire hereof or breastfeeding.

ACR, albumin creatinine ratio; BMI, body mass index; CAG, coronary angiography; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; UA, unstable angina

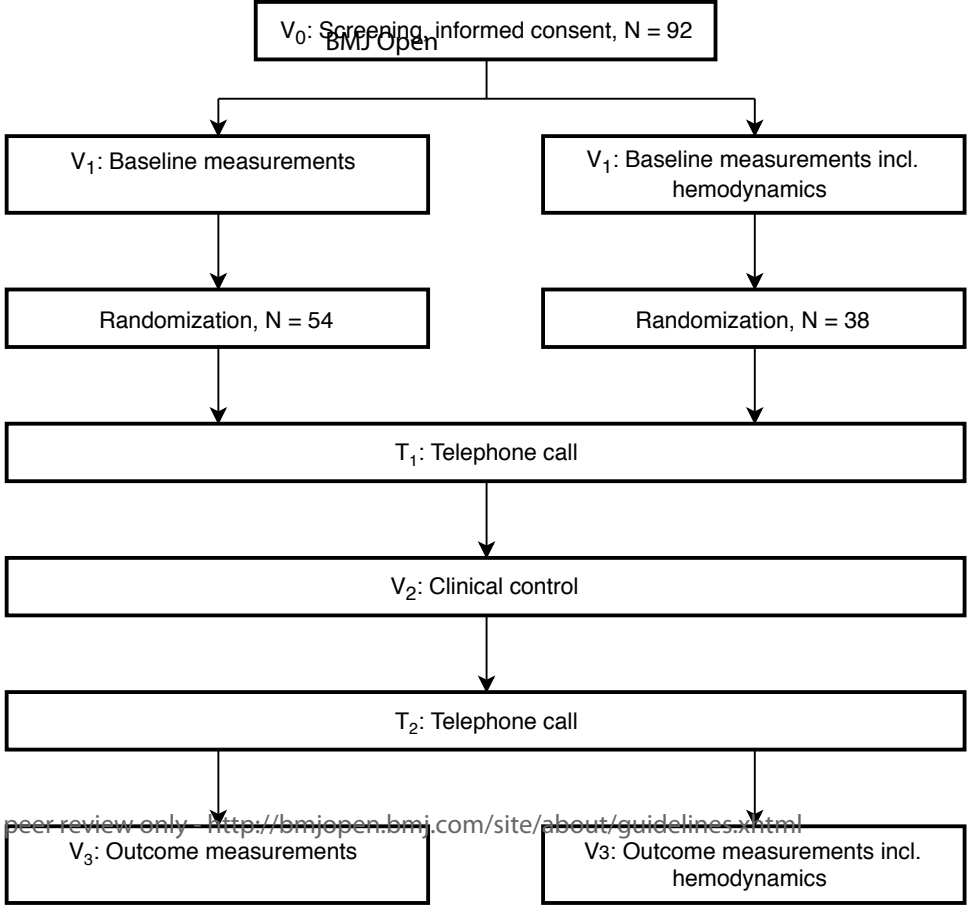
Table 2 Overview of study visits

Visit	V_0	V_1 Randomization	T_1	V_2	T_2	V_3
Time, weeks	-4±1	0±1	2±1	4±1	8±1	12±1
Inclusion/exclusion criteria	X	X				
Medical history	X					
Informed consent	X					
Blood samples, non-fasting	X			X		
Physical examination	X			X		
Adverse events		X	X	X	X	X
Endpoints, Main study						
^{82}Rb -PET (primary endpoint)		X				X
Echocardiography		X				X
Ambulatory BP		X				X
Adipose tissue biopsy		X				X
Oral glucose tolerance test		X				X
Questionnaires		X				X
HRV		X				X
PWA		X				X
^{51}Cr EDTA clearance		X				X
Body composition (DEXA)		X				X
Blood samples, fasting		X				X
Urine samples, fasting		X				X
Endpoints, substudy						
Hemodynamics (key secondary endpoint)		X				X
Accelerometer		X				X

1

Figure 1

Time-line of the study visits



Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,11
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	See note 3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	See note 4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	14
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	See note 5
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return;	See note 6

		laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4-5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4-5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4-5, 11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4
Blinding (masking): emergency	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during	4-5

unblinding		the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	See note 7
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	See note 8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	5
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10

		whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	See note 9
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	See note 10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4,5,11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	See note 11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a (none)
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	See note 12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	10
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 13

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Author notes

1. n/a (not used)
2. 1 (The corresponding author is Sponsor-Investigator)
3. 1,11 (see above)
4. n/a (short, low risk trial)
5. n/a (no specific criteria)
6. n/a (no strategies)
7. n/a (no plans)
8. n/a (short, low risk study)
9. 2 (already approved)
10. n/a (no further modifications are expected)
11. 10 (public access)
12. n/a (standard guideline (Vancouver), no professional writers)
13. n/a (attached in seperate file)

BMJ Open

Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

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Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)

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ABSTRACT

Introduction: A diagnosis of type 2 diabetes (T2D) more than doubles the risk of cardiovascular disease (CVD), with heart failure (HF) being one of the most common complications with a severe prognosis. The landmark EMPA-REG study demonstrated that treatment with the sodium glucose cotransporter-2 (SGLT-2) inhibitor empagliflozin rapidly and significantly reduces CVD mortality and admission rates for HF. However, the mechanisms behind this reduction in clinical events are unknown.

The current study was designed to investigate the effects of the SGLT-2 inhibitor empagliflozin on myocardial perfusion and function in patients with T2D and high CVD risk.

Methods and analysis: In this investigator-initiated, randomized, double-blind controlled clinical trial, 92 patients with T2D and established CVD or high CVD risk, will be randomized to treatment with empagliflozin 25 mg or a matching placebo for 13 weeks. The primary outcome measure is change in myocardial flow reserve measured quantitatively by Rubidium-82 position emission tomography (^{82}Rb-PET). In a substudy, invasive hemodynamics at rest and during exercise will be measured at baseline and following the intervention, using right heart catheterization.

Ethics and dissemination: The study protocol (v7, 02/08/2018) has been approved by the Ethics Committee of the Capital Region, Danish Data Protection Board and the Danish Medicines Agency, and it will be monitored according to the Good Clinical Practice (GCP) regulations from the International Conference on Harmonization. The results be submitted to international peer-reviewed journals and be presented at conferences. The data will be made available to the public via EudraCT and www.clinicaltrials.gov.

Trial Registration: ClinicalTrials.gov Identifier: NCT03151343, EudraCT number: 2016-003743-10

ARTICLE SUMMARY

Strengths and limitations of this study:

- Double-blinded, randomized and placebo controlled.
- The use of advanced imaging techniques.
- Single-center
- No hard endpoints, all outcomes are based on surrogates

INTRODUCTION

Type 2 diabetes (T2D) is a significant risk factor for cardiovascular disease (CVD), including heart failure (HF), and improved glycaemic control is only modestly beneficial in reducing macrovascular disease.[1]

In 2008, prompted by concerns that the T2D drug rosiglitazone might increase the risk of myocardial infarction, the U.S. Food & Drug Administration published guidelines effectively mandating large cardiovascular outcome trials for new T2D drugs. Several such trials have been completed or are ongoing. The trials have primarily examined the safety of dipeptidyl peptidase 4 (DPP-IV) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RA) and sodium–glucose cotransporter 2 (SGLT2) inhibitors.[2–8] The DPP-IV inhibitor trial SAVOR-TIMI indicated an increase in the secondary endpoint, hospitalization due to HF with the active comparator, while another DPP-IV trial, TECOS, as well as the newly published CARMELINA did not demonstrate an increased risk with the active drug. The GLP-1 RA trials LEADER and SUSTAIN although with a reduction in the primary MACE endpoint were neutral with regards to risk of HF. Most of the SGLT-2 trials have demonstrated CV benefits[9] via a reduction of the primary endpoint MACE. In the EMPA-REG outcome study, the SGLT-2 inhibitor empagliflozin reduced the risk of CV death by 38% and in contrast to the GLP-1 RA trials, admission due to HF by 35% in a high-risk T2D population.[6,10]

The novel evidence from the SGLT-2 inhibitor trials was surprising, because the treatments only had a modest effect on classical CV risk factors. Indeed, the mechanism behind their profound effect on CV death and risk of HF remains unknown, although several hypotheses have been proposed. An effect reducing the atherosclerotic burden is considered unlikely, because the risk of CV events was reduced within weeks of treatment[11]. Thus, the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is considered to play a significant role in T2D-related CVD[12] as well as in HF with preserved ejection fraction.[13] Furthermore, coronary vascular dysfunction, particularly decreased vasodilator capacity, is a significant predictor for CV death in patients with T2D.[14,15] Notably, T2D patients with preserved coronary vasodilation had the same

low risk of CVD as subjects without diabetes[14]. Experimental studies have shown that SGLT-2 inhibitor treatment significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary arterial fibrosis and improves vasodilatation in diabetic rodents.[16,17] Thus, part of the underlying effect of empagliflozin in EMPA-REG could be driven by improvement in myocardial microcirculation.

A complementary hypothesis has been suggested to explain the early beneficial effects on HF hospitalization found in the EMPA-REG trial.[18] Inhibition of SGLT-2 is associated with increased diuresis, thus the sodium- and volume-reducing effect could favour cardiac loading conditions in patients with T2D with subclinical dysfunctions of the heart. The proposed effects on central hemodynamics, especially right and left heart filling pressures and cardiac output, can be measured directly by right heart catherization. Pulmonary capillary wedge pressure (PCWP), a measure of left heart filling pressure, has previously been shown to be associated with functional capacity among patients with HF.[19] Therefore, investigating the effect of SGLT-2 inhibitor on central hemodynamics during rest and exercise may contribute vital knowledge on the mechanisms behind the markedly reduced risk of admission for HF demonstrated in EMPA-REG and DECLAIRE TIMI [6,8].

The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR) and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac function.[20] Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral glucose uptake[21] through a reduction in glucose toxicity. SGLT-2 inhibitors have been shown to increase the glucagon-insulin ratio, presumably due to the decrease in glucose stimulus.[22] Moreover they induce a small increase in plasma ketone bodies, which some have proposed to be a preferred fuel source for the myocardium.[23] However, others have shown that SGLT-2 inhibitors increase ATP production in mouse model hearts, without increasing ketone oxidation.[24] Markers of inflammation have been associated with the risk of vascular events, independently of traditional risk factors and an effect on markers of inflammation status such as IL-6 and CRP should also be considered.[25] Therefore, further knowledge on the effects of SGLT-2 inhibitors on metabolic parameters, such as biomarkers of inflammation, adipose tissue function and the relationship with parameters on cardiac function in patients with T2D is warranted.

In summary, SGLT2 inhibitors have been shown to reduce CVD in high-risk T2D patients, with several explanatory hypotheses being suggested that remain to be tested in clinical trials.

Hypothesis

Treatment with empagliflozin for 13 weeks improves the myocardial flow reserve (MFR) in patients with T2D and high cardiovascular risk. The duration of 13 weeks was chosen, as the EMPA-REG study showed a clear effect on the primary outcomes at this timepoint.

Objectives

The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by ^{82}Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study, the effect of empagliflozin on key hemodynamic parameters will be measured during right heart catherization at rest and during exercise. Key secondary outcomes include changes in cardiac echocardiographic evaluations of systolic and diastolic functions, functional capacity by accelerometry, and changes in glucose metabolism, plasma ketone bodies and adipose tissue function.

METHODS AND ANALYSIS

Trial design

This is an investigator-initiated, randomized, placebo-controlled, double-blind trial. Participants will be recruited from the T2D and cardiology outpatient clinics at Herlev-Gentofte University Hospital, Steno Diabetes Center Copenhagen and Rigshospitalet, Denmark. Recruitment started in March 2017 and is expected to continue until early 2020.

Study population

The study will include 92 patients who have had the T2D diagnosis for at least 3 months, and with no upper limit to the duration of diabetes, who have either additional CV risk factors or pre-existing CVD. Detailed inclusion and exclusion criteria are listed in Table 1. Eligible patients will be invited for screening, and patients who meet all the criteria will be randomized to the double-blinded treatment. Participants will be considered part of the intention to treat (ITT) group after receiving the first dose of the study medication”

Trial Intervention

Participants will be randomized 1:1 to either empagliflozin 25 mg or matching placebo once daily for 13 weeks. A cross-over design was considered but not implemented, due to the expected high availability of eligible

participants. A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicated a dose-response effect on metabolic outcomes such as urinary glucose excretion, fasting plasma glucose and mean daily plasma glucose, with higher drug doses resulting in lower glucose levels and increased glucose excretion.[26]. In accordance with the EMPA-REG study, no dose-escalation will be performed. The trial medication will be randomized in computer-generated 1:1 blocks of 10 by the study pharmacy (Glostrup Pharmacy, Copenhagen, Denmark). Participants will receive randomization numbers and the corresponding medication containers sequentially. In case of medical emergency, participants can be unblinded individually. The study medicine will be suspended if the participant becomes pregnant or withdraws consent. The investigators may suspend the medication for safety reasons.

Patient and public involvement

The study was designed without involvement from patients or the public. The patients will be offered access to the results of the study, when these become available.

Trial visits and procedures

Visits

A schematic overview of the trial visits is presented in Table 2 and as a flowchart in Figure 1. At the screening visit (V_0), informed consent will be obtained, and patients will be assessed for eligibility based on medical history, including diabetes duration and comorbidities, physical examinations and blood samples. Ineligible participants will be counted as screen failures, and the reason for screen failure will be recorded.

Outcome-related procedures are performed at the baseline (V_1) and after 13 weeks of treatment (V_3). The trial medication will be dispensed when all the procedures pertaining to V_1 have been completed.

After 3 to 6 weeks of treatment, the participants will visit the trial site for blood tests and a physical examination (V_2). Participant status and compliance as well as adverse events will be assessed at V_2, V_3 and during two phone calls (T_1 and T_2).

^{82}Rb-PET

Myocardial perfusion and myocardial flow reserve (MFR) will be measured using cardiac ^{82}Rb-PET, which allows for flow quantification in absolute terms. To determine the MFR, measurements will be performed at rest and during adenosine-induced stress. A standard clinical protocol will be used. Participants will be scanned in the supine position using a Siemens Biograph mCT/PET 128-slice scanner (Siemens Medical Solutions, Knoxville, USA). Low-dose non-contrast CT will be acquired for attenuation correction as well as measurement of cardiac adipose tissue volume. Approximately 1100 MBq of ^{82}Rb will be obtained from a CardioGen-82 Sr-82/Rb-82 generator (Bracco Diagnostics, Inc., Princeton, New Jersey, USA) and intravenously infused with a constant flowrate of 50 mL/min. List-mode 3D data acquisition will begin with the tracer infusion and continue for 7 minutes. Static and ECG-gated images will be reconstructed with a 2.5-minute delay to allow ^{82}Rb to clear from the blood pool. Maximal hyperaemia will be induced with adenosine infused at 140 µg/kg/min for 6 minutes. After 2.5 minutes of adenosine infusion, intravenous ^{82}Rb infusion and list-mode acquisition will follow the same protocol as for rest. Myocardial blood perfusion quantification (in mL/min/g) will be performed using Cedars-Sinai QGS+QPS 2015.6 software (Cedars-Sinai Medical Center), which is based on a single-compartment model for Rb-82 tracer kinetics.

Hemodynamics sub-study

In a substudy, 38 participants randomly selected from the primary study population will perform a graded exercise test until maximal exertion using a supine ergometer with simultaneous invasive hemodynamic measurements. Using local anaesthesia, A Swan-Ganz catheter will be placed into the pulmonary artery via the right internal jugular vein. The following hemodynamic variables will be assessed: pulmonary capillary wedge pressure (PCWP), cardiac output (CO) using thermodilution, central venous pressure (CVP and pulmonary artery pressure (PAP). Following measurements at rest, participants will be transferred to a supine bicycle ergometer, and the measurements will be repeated (leg-raise situation). The participant will be instructed to pedal at 60 rpm and the workload is incrementally increased at steps of 10watts. Measurements during exercise will be obtained at 25 watts and during peak exercise. Blood sampled from the pulmonary vein will be obtained at rest, 25 watts and at peak exercise for analyses of lactate, oxygen saturation and other blood gas variables.

Echocardiography

The following echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a') velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal speckle tracking) will be assessed via 2D echocardiography. The longitudinal tissue velocity of the LV will be assessed by averaging myocardial velocities and displacement of the mitral annular position in the septal, lateral, inferior, posterior and anterior wall of the LV. Diastolic function will be assessed according to EAE/ASE recommendations.

Cr-51 EDTA

The Cr-51 EDTA method will be used to measure glomerular filtration rate (GFR). A single intravenous administration of 3.7MBq of Cr-51 EDTA is given. A venous sample is drawn four hours after administration.

Accelerometry

A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the accelerometer continuously for 7 days, except for bathing and swimming.

Ambulatory blood pressure

24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every hour during the night.

Adipose tissue biopsy

Biopsies will be obtained from the abdominal subcutaneous tissue lateral to the umbilicus during a fasting state using the Bergstrom needle technique. Following dissection, the biopsies are washed and snap frozen in liquid nitrogen and stored at -80°C. Total mRNA will be extracted from adipose tissue with commercially available lipid tissue kits to measure the mRNA expression of biomarkers of interest. Protein expression will

be analysed using Western blots. Quantification of inflammatory cells will be performed according to validated histological procedures. Fibrosis levels will be measured using the Sirius Red stain.

Oral Glucose Tolerance Test (OGTT)

The fasting participant will drink a solution of 75g of glucose. Blood will be drawn at time points 0, 30, 60 and 120 minutes, and measurements of plasma glucose, C-peptide and plasma insulin will be taken. Frozen aliquots for measurement of glucagon will be kept for a later sub-study.

Questionnaires

Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.

Heart rate variability (HRV)

Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and while performing the Valsalva manoeuvre.

Pulse wave analysis (PWA)

Arterial stiffness will be measured using PWA. The procedure will be performed using a SphygmoCor device (version 7.0, Atcor Medical, Sydney, Australia) at the right radial artery.

Body composition

Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software provides a novel method for differentiating between visceral and subcutaneous abdominal fat.

Urine and blood samples, fasting

Blood will be drawn in the fasting state, and patients will provide a sample of first morning urine. Standard blood analyses will be performed immediately after sampling. Urine samples for measurement of albumin/creatinine ratio and blood samples for specialized biomarkers will be stored at -80°C until batch analysis can be performed. Blood analysis will include inflammatory biomarkers such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), cardiospecific biomarkers such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TNT), as well as markers of adipocyte function, including adiponectin and leptin.

ENDPOINTS

Primary outcome measurement

The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow reserve MFR assessed by ^{82}Rb-PET

Key sub-study measurement

The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts

Secondary outcome measurements

The effect of empagliflozin compared with placebo on the following:

- Global left and right heart function
- Renal function by Cr51-EDTA plasma clearance
- Cardiac adipose tissue volume
- PCWP at peak exercise, corrected to body weight
- Plasma levels of NT-proBNP , MR-proANP, MR-proADM, GAL-3, hsTNT, GDF-15, PIGF, sFlt-1, FFA, adiponectin, leptin, TNF-α, IL-6, MCP-1, MAC-1, COLL-A1, endothelin-1 and FGF-21.
- Daily activity levels
- Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
- Ambulatory systolic and diastolic blood pressures
- Quality of life assessed by the Minnesota Living with Heart Failure and the EuroQol EQ-5D-5L questionnaires
- Plasma beta-hydroxy butyrate levels
- Urine albumin/creatinine ratio
- Adipose tissue fibrosis, mRNA and protein expression of TNF-α, adiponectin, IL-6, COL1-A1, MAC-1, FGF-21, and monocyte chemoattractant protein 1 (MCP1)

STATISTICAL ANALYSIS

SAMPLE SIZE

Primary endpoint

The primary endpoint is change in MFR. In a previous study on a comparable population, the SD of measurements of MFR by ^{82}Rb PET was 0.8. Assuming a clinically relevant difference between the treatment and placebo groups after 6 months of 0.5 in MFR,[27] a sample size of 41:41 (empagliflozin: placebo) can be detected with 80% power and a two-sided significance level of 5%. In all, 92 patients will be randomized to allow for a 10% drop-out rate from the intention-to-treat (ITT) population.

Sub-study key endpoint

Based on recent experiments in a comparable study population,[28] a difference of 5 mmHg in PCWP during exercise was considered clinically significant. The SD of measurements of PCWP was 5 mmHg; thus, a sample size of 16:16 is required with a power of 80% and a two-sided significance level of 5% to detect a difference of 5 mmHg between groups. In total 38 patients will be included in the sub-study to allow for a 15% drop-out rate.

In the ITT population, none of the randomized participants will be excluded, and the patients will be analysed according to the randomization group. The per-protocol population will consist of all the patients who completed the study with a documented valid baseline and a final-week assessment of the primary objective without any major protocol violations. Analysis of the primary outcome parameter will focus on a change in global MBF from the baseline to week 13 between the treatment and control groups. The primary analysis will be based on the ITT population. The primary outcome measure will be analysed using ANCOVA with treatment as a factor and the baseline value as a covariate. The model will include the prespecified covariates age and gender. Missing data will be estimated using the maximum likelihood method. Normally distributed variables will be presented as mean ±SD, and non-parametric statistics or appropriate log transformation will be performed if an assumption of normality is not met. After log transformation, the variable will be further tested for normality distribution as indicated. A two-tailed *p* value of less than 0.05 will be considered statistically significant.

Comparisons between the treatment groups will be performed by an unpaired two sample *t*-test, Mann-Whitney test or χ2 - test as appropriate.

Data management

Source data will be recorded in the patient record or in case report forms (CRF). The requirements for entering source data directly into the CRF are that the data are obtained directly from the patient either by clinical assessment, interview or point-of-care systems with no printout function and that no more reliable forms of data capture are available. Medical history, height and weight are examples of such data.

A CRF will be constructed for data capture. Data will be stored in coded form for 5 years according to recommendations from the Danish Data Protection Agency; thereafter, data will be transferred to the Danish Data Archives.

Study medication

Name: Jardiance® (empagliflozin) or a visually identical matching placebo

Pharmaceutical Form: Tablet for oral use

Pharmacological Dosage: Jardiance® or a placebo will be introduced at a dose of 25 mg/day.

Intake of the tablet can be done at any time during the day; however, it is recommended that the time of intake be consistent from day to day.

Side effects: Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis, vulvovaginitis

Shipping and packing: All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

Randomization: Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned consecutively, and patients will retain these numbers following randomization.

Concomitant medication

Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving

optimal T2D therapy, and during the trial, T2D and CV risk or disease will be managed according to the best available evidence.

ETHICS AND DISSEMINATION

The study, which will be conducted in accordance with the latest revision of the Helsinki Declaration, EU directive on GCP and ICH-GCP guidelines, has been approved by the Regional Scientific Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. The study is registered at clinicaltrials.gov and monitored by the GCP unit at Bispebjerg University Hospital. The results of the project will be submitted to international peer-reviewed journals regardless of their outcome, and the data will be made available to the public via EudraCT and www.clinicaltrials.gov. Furthermore, the results will be presented at conferences as abstracts and posters.

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Author Contributions:

CK, MS, FG, AK, PH, JF, BZ, SI and MJ conceived the study and participated in its design, planning and coordination. CK, MS, MW, PG, PR, NB and MJ are responsible for the inclusion and examination of patients at Herlev-Gentofte and Rigshospitalet University Hospital. AK and PH are responsible for the ^{82}Rb-PET measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.

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Competing interests:

PR has received consultancy and/or speaking fees (to his institution) from AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, MSD, Novo Nordisk and Sanofi Aventis and has received institutional research grants from AbbVie, AstraZeneca and Novo Nordisk.

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Table 1 Inclusion and exclusion criteria

Inclusion criteria

- T2D (WHO criteria), diagnosed at least 3 months before screening, and with no upper limit to duration
- For patients on background therapy: No change in anti-diabetic therapy within 30 days prior to baseline
- Hba1c of ≥ 48 mmol/L and ≤ 86 mmol/L at screening for patients on background therapy or Hba1c of ≥ 48 mmol/L and ≤ 75 mmol/L at screening for drug-naïve patients
- Age ≥ 18 years
- BMI ≤ 45 kg/m
- Negative pregnancy test (fertile women). Fertile women must use safe contraceptives (spiral, hormonal contraceptives) for the duration of the study
- Able to understand the written patient information and to give informed consent
- High cardiovascular risk, defined as at least one of the following:
  - ACR ≥ 30 mg/g
  - NT-proBNP ≥ 70 pg/mL
  - Confirmed history of myocardial infarction > 2 months prior to baseline
  - Heart failure according to Framingham Heart Failure Criteria
  - Discharged from hospital with a documented diagnosis of UA ≤ 12 months prior to baseline
  - Evidence of coronary artery disease by CAG in one or more major coronary arteries OR at least one of the following: a positive noninvasive stress test, or a positive stress echocardiography showing regional systolic wall motion abnormalities, or a positive scintigraphic test showing stress-induced ischemia
  - History of ischemic or hemorrhagic stroke > 2 months prior to informed consent
  - Presence of peripheral artery disease such as: previous limb angioplasty, stenting or bypass surgery; or previous limb or foot amputation due to circulatory insufficiency; or angiographic evidence of significant (> 50%) peripheral artery stenosis in at least one limb; or evidence from a non-invasive measurement of significant (>50% or as reported as hemodynamically significant) peripheral artery stenosis in at least one limb; or ankle brachial index of < 0.9

Exclusion criteria

- Allergic to the study medication
- Treatment with SGLT-2 inhibitor within 1 month prior to baseline
- Impaired kidney function, eGFR ≤ 30 ml/min
- Severe liver insufficiency (Child-Pugh class C)
- ECG showing malign ventricular arrhythmia or prolonged QT-interval (>500ms)
- Untreated clinically significant heart valve disease
- Planned cardiac surgery or angioplasty within 3 months.
- Myocardial infarction (MI) ≤ 30 days prior to baseline
- Percutaneous coronary intervention (PCI) ≤ 4 weeks prior to baseline
- History of coronary artery bypass graft (CABG) ≤ 8 weeks prior to enrollment
- Prior history of heart transplantation
- Unstable angina, known severe left main coronary artery stenosis, severe heart failure, uncontrolled

Table 2 Overview of study visits

Visit	V_0	V_1 Randomization	T_1	V_2	T_2	V_3
Time, weeks	-4±1	0±1	2±1	4±1	8±1	12±1
Inclusion/exclusion criteria	X	X				
Medical history	X					
Informed consent	X					
Blood samples, non-fasting	X			X		
Physical examination	X			X		
Adverse events		X	X	X	X	X
Endpoints, Main study						
^{82}Rb -PET (primary endpoint)		X				X
Echocardiography		X				X
Ambulatory BP		X				X
Adipose tissue biopsy		X				X
Oral glucose tolerance test		X				X
Questionnaires		X				X
HRV		X				X
PWA		X				X
^{51}Cr EDTA clearance		X				X
Body composition (DEXA)		X				X
Blood samples, fasting		X				X
Urine samples, fasting		X				X
Endpoints, substudy						
Hemodynamics (key secondary endpoint)		X				X
Accelerometer		X				X

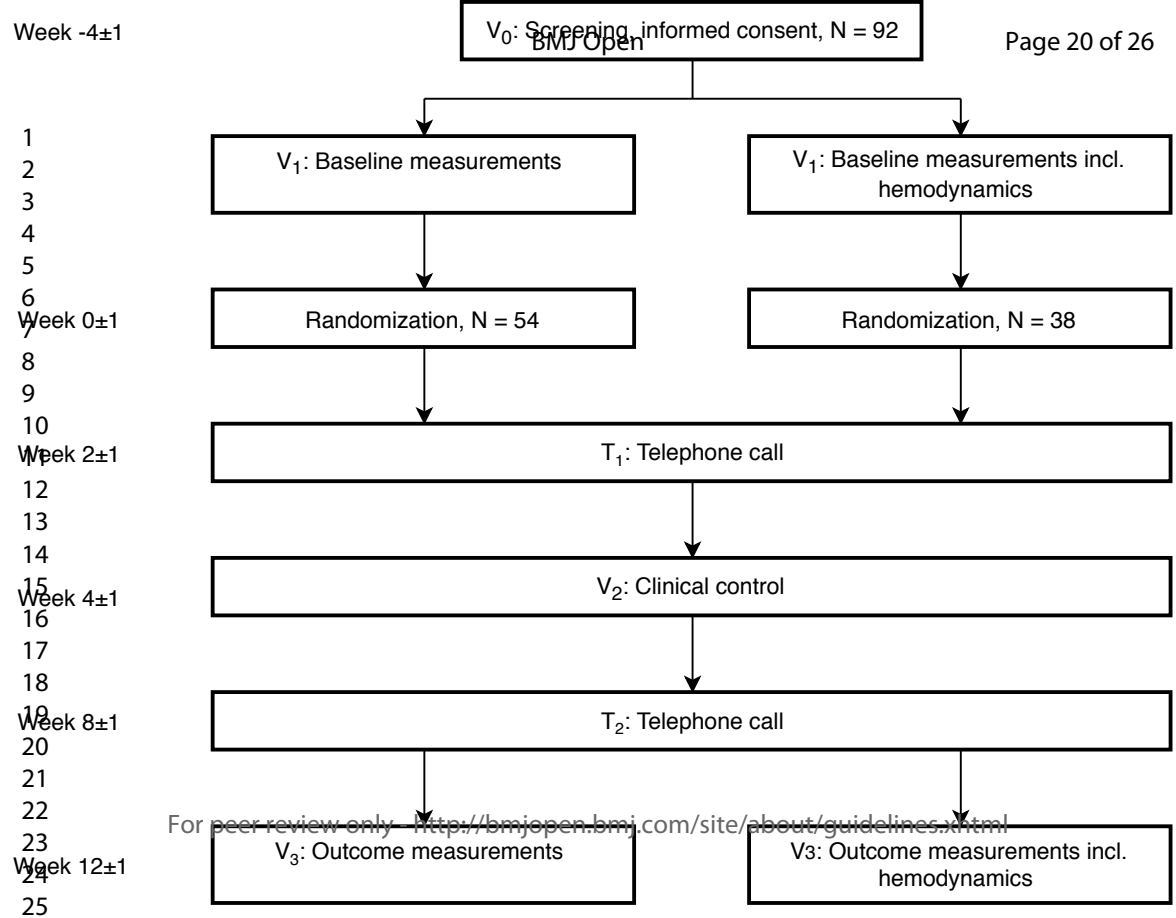
1

2

1

Figure 1

Time-line of the study visits



Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,11
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	See note 3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	See note 4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	14
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	See note 5
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return;	See note 6

		laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4-5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4-5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4-5, 11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4
Blinding (masking): emergency	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during	4-5

unblinding		the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	See note 7
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	See note 8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	5
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10

		whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	See note 9
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	See note 10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4,5,11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	See note 11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a (none)
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	See note 12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	10
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 13

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Author notes

1. n/a (not used)
2. 1 (The corresponding author is Sponsor-Investigator)
3. 1,11 (see above)
4. n/a (short, low risk trial)
5. n/a (no specific criteria)
6. n/a (no strategies)
7. n/a (no plans)
8. n/a (short, low risk study)
9. 2 (already approved)
10. n/a (no further modifications are expected)
11. 10 (public access)
12. n/a (standard guideline (Vancouver), no professional writers)
13. n/a (attached in seperate file)