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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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Manuscripts

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8 on myocardial perfusion, function and metabolism in type 2 diabetes
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10 patients at high cardiovascular risk (The SIMPLE trial)
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52 **ABSTRACT**
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4 **Introduction:** A diagnosis of type 2 diabetes (T2D) more than doubles the risk of cardiovascular disease
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6 (CVD), with heart failure (HF) being one of the most common complications with a severe prognosis.
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10 The landmark EMPA-REG study demonstrated that treatment with the sodium glucose cotransporter-2
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12 (SGLT-2) inhibitor empagliflozin rapidly and significantly reduces CVD mortality and admission rates
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14 for HF. However, the mechanisms behind this reduction in clinical events are unknown.
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17 The current study was designed to investigate the effects of the SGLT-2 inhibitor empagliflozin on
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19 myocardial perfusion and function in patients with T2D and high CVD risk.
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22 **Methods and analysis:** In this investigator-initiated, randomized, double-blind controlled clinical trial,
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24 92 patients with T2D and established CVD or high CVD risk, will be randomized to treatment with
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26 empagliflozin 25 mg or a matching placebo for 13 weeks. The primary outcome measure is change in
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28 myocardial flow reserve measured quantitatively by Rubidium-82 position emission tomography (⁸²Rb-
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30 PET). In a substudy, invasive hemodynamics at rest and during exercise will be measured at baseline
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32 and following the intervention, using right heart catheterization.
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38 **Ethics and dissemination:** The study protocol (v7, 02/08/2018) has been approved by the Ethics
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40 Committee of the Capital Region, Danish Data Protection Board and the Danish Medicines Agency, and
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42 it will be monitored according to the Good Clinical Practice (GCP) regulations from the International
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44 Conference on Harmonization.
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48 **Trial Registration:** ClinicalTrials.gov Identifier: NCT03151343
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54 ARTICLE SUMMARY

55 Strengths and limitations of this study:

- 56 • Double-blinded, randomized and placebo controlled.
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- 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 benefits⁹ 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 CVD¹⁰ 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 diabetes¹². 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.

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

21 The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR)
22 and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines
23 mediating ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental
24 impact on cardiac function.¹⁸ Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly
25 increasing peripheral glucose uptake¹⁹ through a reduction in glucose toxicity. Moreover they induce a small
26 increase in plasma ketone bodies, which may serve as a preferred fuel source for the myocardium.²⁰
27 Therefore, further knowledge on the effects of SGLT-2 inhibitors on metabolic parameters, including
28 dysfunctional adipose tissue and the relationship with parameters on cardiac function in patients with T2D
29 is warranted.

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

42 Hypothesis

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

48 Objectives

50 The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by
51 ⁸²Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study,
52 the effect of empagliflozin on key hemodynamic parameters will be measured during right heart
53 catheterization at rest and during exercise. Key secondary outcomes include changes in cardiac
54 echocardiographic evaluations of systolic and diastolic functions, functional capacity by accelerometry, and
55 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

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4 Myocardial perfusion and myocardial flow reserve (MFR) will be measured using cardiac ^{82}Rb -PET, which
5 allows for flow quantification in absolute terms. To determine the MFR, measurements will be performed
6 at rest and during adenosine-induced stress. A standard clinical protocol will be used. Participants will be
7 scanned in the supine position using a Siemens Biograph mCT/PET 128-slice scanner (Siemens Medical
8 Solutions, Knoxville, USA). Low-dose non-contrast CT will be acquired for attenuation correction as well as
9 measurement of cardiac adipose tissue volume. Approximately 1100 MBq of ^{82}Rb will be obtained from a
10 CardioGen-82 Sr-82/Rb-82 generator (Bracco Diagnostics, Inc., Princeton, New Jersey, USA) and
11 intravenously infused with a constant flowrate of 50 mL/min. List-mode 3D data acquisition will begin with
12 the tracer infusion and continue for 7 minutes. Static and ECG-gated images will be reconstructed with a
13 2.5-minute delay to allow ^{82}Rb to clear from the blood pool. Maximal hyperaemia will be induced with
14 adenosine infused at 140 $\mu\text{g}/\text{kg}/\text{min}$ for 6 minutes. After 2.5 minutes of adenosine infusion, intravenous
15 ^{82}Rb infusion and list-mode acquisition will follow the same protocol as for rest. Myocardial blood perfusion
16 quantification (in mL/min/g) will be performed using Cedars-Sinai QGS+QPS 2015.6 software (Cedars-Sinai
17 Medical Center), which is based on a single-compartment model for Rb-82 tracer kinetics.
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31 **Hemodynamics sub-study**

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

55 Echocardiographic measurements will be obtained using 3D and 2D imaging at rest: left ventricular
56 ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial
57 volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric
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4 relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber,
5 two-chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a')
6 velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal
7 speckle tracking) will be assessed via 2D echocardiography.
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13 **Cr-51 EDTA**

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

21 A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the
22 accelerometer continuously for 7 days, except for bathing and swimming.
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26 **Ambulatory blood pressure**

27 24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM
28 Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and
29 every hour during the night.
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35 **Adipose tissue biopsy**

36 Biopsies will be obtained from the abdominal subcutaneous tissue during a fasting state using the
37 Bergstrom needle technique. Total mRNA will be extracted from adipose tissue with commercially
38 available lipid tissue kits to measure mRNA expression of adipocytokines. Quantification of inflammatory
39 cells will be performed according to validated histological procedures.
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46 **Oral Glucose Tolerance Test (OGTT)**

47 The fasting participant will drink a solution of 75g of glucose. Blood will be drawn at time points 0, 30,
48 60 and 120 minutes, and measurements of plasma glucose, C-peptide and plasma insulin will be taken.
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52 **Questionnaires**

53 Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.
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57 **Heart rate variability (HRV)**

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4 Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be
5 measured at rest, while rising from a lying position to standing upright, during deep in- and expiration
6 and while performing the Valsalva maneuver.
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10 11 **Pulse wave analysis (PWA)**

12 Arterial stiffness will be measured using PWA. The procedure will be performed using a SphygmoCor
13 device (version 7.0, Atcor Medical, Sydney, Australia) at the right radial artery.
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17 18 **Body composition**

19 Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA
20 scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software
21 provides a novel method for differentiating between visceral and subcutaneous abdominal fat.
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26 27 **Urine and blood samples, fasting**

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

36 37 **Primary outcome measurement**

38 The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow
39 reserve MFR assessed by ⁸²Rb-PET
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42 **Key sub-study measurement**

43 The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts
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47 48 **Secondary outcome measurements**

49 The effect of empagliflozin compared with placebo on the following:
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- 51 - Global left and right heart function
- 52 - Renal function by Cr51-EDTA plasma clearance
- 53 - Cardiac adipose tissue volume
- 54 - PCWP at peak exercise, corrected to body weight
- 55 - Plasma NT-proBNP concentrations
- 56 - Daily activity levels
- 57 - Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
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- 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 \pm 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.

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4 Comparisons between the treatment groups will be performed by an unpaired two sample *t*-test, Mann-
5 Whitney test or χ^2 - test as appropriate.
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8 9 Data management

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

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

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30 *Pharmaceutical Form:* Tablet for oral use

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

33 Intake of the tablet can be done at any time during the day; however, it is recommended that the time of
34 intake be consistent from day to day.
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37 *Side effects:* Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or
38 sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis,
39 vulvovaginitis
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42 *Shipping and packing:* All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

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

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51 Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant
52 medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving
53 optimal T2D therapy, and during the trial, T2D and CV risk or disease will be managed according to the best
54 available evidence.
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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 ⁸²Rb-PET measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.

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Competing interests:

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4 PR has received consultancy and/or speaking fees (to his institution) from AbbVie, Astellas, AstraZeneca,
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11 REFERENCES

- 12 1. Turner R. Intensive blood-glucose control with sulphonylureas or insulin compared with
13 conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33).
14 *Lancet*. 1998;352(9131):837-853. doi:10.1016/S0140-6736(98)07019-6
- 15 2. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and Cardiovascular Outcomes in Patients with
16 Type 2 Diabetes Mellitus. *N Engl J Med*. 2013;369(14):1317-1326. doi:10.1056/NEJMoa1307684
- 17 3. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type
18 2 Diabetes. *N Engl J Med*. 2015;373(3):232-242. doi:10.1056/NEJMoa1501352
- 19 4. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute
20 Coronary Syndrome. *N Engl J Med*. 2015;373(23):2247-2257. doi:10.1056/NEJMoa1509225
- 21 5. White WB, Cannon CP, Heller SR, et al. Alogliptin after Acute Coronary Syndrome in Patients with
22 Type 2 Diabetes. *N Engl J Med*. 2013;369(14):1327-1335. doi:10.1056/NEJMoa1305889
- 23 6. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in
24 Type 2 Diabetes. *N Engl J Med*. 2015;373(22):2117-2128. doi:10.1056/NEJMoa1504720
- 25 7. Guthrie R. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *Postgrad Med*.
26 2018;130(2):149-153. doi:10.1080/00325481.2018.1423852
- 27 8. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N*
28 *Engl J Med*. 2018;NEJMoa1812389. doi:10.1056/NEJMoa1812389
- 29 9. Cefalu WT, Kaul S, Gerstein HC, et al. Cardiovascular outcomes trials in type 2 diabetes: Where Do
30 We Go From Here? Ref lections From a Diabetes Care Editors' Expert Forum. *Diabetes Care*.
31 2018;41(1):14-31. doi:10.2337/dci17-0057
- 32 10. Avogaro A, Albiero M, Menegazzo L, De Kreutzenberg S, Fadini GP. Endothelial dysfunction in
33 diabetes: The role of reparatory mechanisms. *Diabetes Care*. 2011;34(SUPPL. 2):285-290.
34 doi:10.2337/dc11-s239
- 35 11. Srivaratharajah K, Coutinho T, Dekemp R, et al. Reduced myocardial flow in heart failure patients
36 with preserved ejection fraction. *Circ Hear Fail*. 2016;9(7).
37 doi:10.1161/CIRCHEARTFAILURE.115.002562
- 38 12. Murthy VL, Naya M, Foster CR, et al. Association between coronary vascular dysfunction and cardiac
39 mortality in patients with and without diabetes mellitus. *Circulation*. 2012;126(15):1858-1868.
40 doi:10.1161/CIRCULATIONAHA.112.120402
- 41 13. Cortigiani L, Rigo F, Gherardi S, et al. Additional Prognostic Value of Coronary Flow Reserve in
42 Diabetic and Nondiabetic Patients With Negative Dipyridamole Stress Echocardiography by Wall
43 Motion Criteria. *J Am Coll Cardiol*. 2007;50(14):1354-1361. doi:10.1016/j.jacc.2007.06.027
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14. Lin B, Koibuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol*. 2014;13(1). doi:10.1186/s12933-014-0148-1
15. Oelze M, Kröller-Schön S, Welschof P, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. *PLoS One*. 2014;9(11):e112394. doi:10.1371/journal.pone.0112394
16. Sattar N, McLaren J, Kristensen SL, Preiss D, McMurray JJ. SGLT2 Inhibition and cardiovascular events: why did EMPA-REG Outcomes surprise and what were the likely mechanisms? *Diabetologia*. 2016:1-7. doi:10.1007/s00125-016-3956-x
17. Emil W, David K, A. BB, et al. Resting and exercise haemodynamics in relation to six-minute walk test in patients with heart failure and preserved ejection fraction. *Eur J Heart Fail*. 2017;20(4):715-722. doi:10.1002/ejhf.976
18. Sharma S, Adroque J V, Golfman L, et al. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J*. 2004;18(14):1692-1700. doi:10.1096/fj.04-2263com
19. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest*. 2014;124(2):499-508. doi:10.1172/JCI72227.uptake
20. Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A “Thrifty Substrate” Hypothesis. *Diabetes Care*. 2016;(March):dc160330. doi:10.2337/dc16-0330
21. von Scholten BJ, Hasbak P, Christensen TE, et al. Cardiac 82Rb PET/CT for fast and non-invasive assessment of microvascular function and structure in asymptomatic patients with type 2 diabetes. *Diabetologia*. 2016;59(2):371-378. doi:10.1007/s00125-015-3799-x
22. Andersen MJ, Ersbøll M, Axelsson A, et al. Sildenafil and diastolic dysfunction after acute myocardial infarction in patients with preserved ejection fraction: The sildenafil and diastolic dysfunction after acute myocardial infarction (SIDAMI) trial. *Circulation*. 2013;127(11):1200-1208. doi:10.1161/CIRCULATIONAHA.112.000056

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 malignant ventricular arrhythmia or prolonged QT-interval (>500 ms)
- ▶ 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 > 1 first degree atrioventricular block in the absence of a functioning pacemaker

Table 2 Overview of study visits

Visit	V ₀	V ₁	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.

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

1	sponsor contact			
2	information			
3				
4	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	See note
5	responsibilities:		collection, management, analysis, and interpretation of data; writing	3
6	sponsor and funder		of the report; and the decision to submit the report for publication,	
7			including whether they will have ultimate authority over any of these	
8			activities	
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12	Roles and	#5d	Composition, roles, and responsibilities of the coordinating centre,	n/a
13	responsibilities:		steering committee, endpoint adjudication committee, data	
14	committees		management team, and other individuals or groups overseeing the	
15			trial, if applicable (see Item 21a for data monitoring committee)	
16				
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18				
19	Background and	#6a	Description of research question and justification for undertaking the	2-4
20	rationale		trial, including summary of relevant studies (published and	
21			unpublished) examining benefits and harms for each intervention	
22				
23				
24	Background and	#6b	Explanation for choice of comparators	See note
25	rationale: choice of			4
26	comparators			
27				
28				
29	Objectives	#7	Specific objectives or hypotheses	4
30				
31				
32	Trial design	#8	Description of trial design including type of trial (eg, parallel group,	4
33			crossover, factorial, single group), allocation ratio, and framework	
34			(eg, superiority, equivalence, non-inferiority, exploratory)	
35				
36				
37	Study setting	#9	Description of study settings (eg, community clinic, academic	4
38			hospital) and list of countries where data will be collected. Reference	
39			to where list of study sites can be obtained	
40				
41				
42	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable,	14
43			eligibility criteria for study centres and individuals who will perform	
44			the interventions (eg, surgeons, psychotherapists)	
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48	Interventions:	#11a	Interventions for each group with sufficient detail to allow	4-5
49	description		replication, including how and when they will be administered	
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51				
52	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a	See note
53	modifications		given trial participant (eg, drug dose change in response to harms,	5
54			participant request, or improving / worsening disease)	
55				
56				
57	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any	See note
58	adherence		procedures for monitoring adherence (eg, drug tablet return;	6
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		laboratory tests)	
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3	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or
4	concomitant care		prohibited during the trial
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6	Outcomes	#12	Primary, secondary, and other outcomes, including the specific
7			measurement variable (eg, systolic blood pressure), analysis metric
8			(eg, change from baseline, final value, time to event), method of
9			aggregation (eg, median, proportion), and time point for each
10			outcome. Explanation of the clinical relevance of chosen efficacy
11			and harm outcomes is strongly recommended
12			
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16	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins
17			and washouts), assessments, and visits for participants. A schematic
18			diagram is highly recommended (see Figure)
19			
20			
21	Sample size	#14	Estimated number of participants needed to achieve study objectives
22			and how it was determined, including clinical and statistical
23			assumptions supporting any sample size calculations
24			
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26	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach
27			target sample size
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30	Allocation: sequence	#16a	Method of generating the allocation sequence (eg, computer-
31	generation		generated random numbers), and list of any factors for stratification.
32			To reduce predictability of a random sequence, details of any
33			planned restriction (eg, blocking) should be provided in a separate
34			document that is unavailable to those who enrol participants or
35			assign interventions
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40	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
41	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
42	mechanism		describing any steps to conceal the sequence until interventions are
43			assigned
44			
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47	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
48	implementation		participants, and who will assign participants to interventions
49			
50			
51	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
52			participants, care providers, outcome assessors, data analysts), and
53			how
54			
55			
56	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible, and
57	emergency		procedure for revealing a participant's allocated intervention during
58			
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1	unblinding		the trial	
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3	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
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12	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
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17	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
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24	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
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30	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
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33				
34	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
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39	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
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47	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
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52	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
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58	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10
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		whether the process will be independent from investigators and the sponsor	
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4	Research ethics approval	#24 Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	See note 9
5			
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7			
8	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
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14	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
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18	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
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22	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
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27	Declaration of interests	#28 Financial and other competing interests for principal investigators for the overall trial and each study site	11
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31	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
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36	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)
37			
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40	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
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47	Dissemination policy: authorship	#31b Authorship eligibility guidelines and any intended use of professional writers	See note 12
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51	Dissemination policy: reproducible research	#31c Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	10
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55	Informed consent materials	#32 Model consent form and other related documentation given to participants and authorised surrogates	See note 13
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1 Biological specimens #33 Plans for collection, laboratory evaluation, and storage of biological n/a
2 specimens for genetic or molecular analysis in the current trial and
3 for future use in ancillary studies, if applicable
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7 Author notes

- 9 1. n/a (not used)
- 11 2. 1 (The corresponding author is Sponsor-Investigator)
- 13 3. 1,11 (see above)
- 15 4. n/a (short, low risk trial)
- 17 5. n/a (no specific criteria)
- 19 6. n/a (no strategies)
- 21 7. n/a (no plans)
- 23 8. n/a (short, low risk study)
- 25 9. 2 (already approved)
- 27 10. n/a (no further modifications are expected)
- 29 11. 10 (public access)
- 31 12. n/a (standard guideline (Vancouver), no professional writers)
- 33 13. n/a (attached in seperate file)

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37 3.0. This checklist was completed on 11. January 2019 using <https://www.goodreports.org/>, a tool made by the
39 [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-029098.R1
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Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Diabetic nephropathy & vascular disease < DIABETES & ENDOCRINOLOGY, RADIOLOGY, CARDIOLOGY, Cardiovascular imaging < RADIOLOGY & IMAGING

SCHOLARONE™
Manuscripts

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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Word count: 3147

Mesh terms: Diabetes Mellitus, Type 2; Sodium-Glucose Transporter 2 Inhibitors, Cardiovascular Diseases, Myocardial Perfusion Imaging, Cardiac Catheterization

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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

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4 trials for new T2D drugs. Several such trials have been completed or are ongoing. The trials have primarily
5 examined the safety of dipeptidyl peptidase 4 (DPP-IV) inhibitors, glucagon-like peptide-1 receptor agonists
6 (GLP-1 RA) and sodium–glucose cotransporter 2 (SGLT2) inhibitors.^{2–8} The DPP-IV inhibitor trial SAVOR-TIMI
7 indicated an increase in the secondary endpoint, hospitalization due to HF with the active comparator, while
8 another DPP-IV trial, TECOS, as well as the newly published CARMELINA did not demonstrate an increased risk
9 with the active drug. The GLP-1 RA trials LEADER and SUSTAIN although with a reduction in the primary MACE
10 endpoint were neutral with regards to risk of HF. Most of the SGLT-2 trials have demonstrated CV benefits⁹ via
11 a reduction of the primary endpoint MACE. In the EMPA-REG outcome study, the SGLT-2 inhibitor empagliflozin
12 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
13 high-risk T2D population.^{6,10}

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21 The novel evidence from the SGLT-2 inhibitor trials was surprising, because the treatments only had a modest
22 effect on classical CV risk factors. Indeed, the mechanism behind their profound effect on CV death and risk of
23 HF remains unknown, although several hypotheses have been proposed. An effect reducing the atherosclerotic
24 burden is considered unlikely, because the risk of CV events was reduced within weeks of treatment¹¹. Thus,
25 the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is
26 considered to play a significant role in T2D-related CVD¹² as well as in HF with preserved ejection fraction.¹³
27 Furthermore, coronary vascular dysfunction, particularly decreased vasodilator capacity, is a significant
28 predictor for CV death in patients with T2D.^{14,15} Notably, T2D patients with preserved coronary vasodilation
29 had the same low risk of CVD as subjects without diabetes¹⁴. Experimental studies have shown that SGLT-2
30 inhibitor treatment significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary
31 arterial fibrosis and improves vasodilatation in diabetic rodents.^{16,17} Thus, part of the underlying effect of
32 empagliflozin in EMPA-REG could be driven by improvement in myocardial microcirculation.

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

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8 The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR)
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10 and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating
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12 ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac
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14 function.²⁰ Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral
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16 glucose uptake²¹ through a reduction in glucose toxicity. Moreover they induce a small increase in plasma
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18 ketone bodies, which may serve as a preferred fuel source for the myocardium.²² Markers of inflammation
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20 have been associated with the risk of vascular events, independently of traditional risk factors and an effect on
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22 inflammation status should also be considered²³. Therefore, further knowledge on the effects of SGLT-2
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24 inhibitors on metabolic parameters, such as biomarkers of inflammation, adipose tissue function and the
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26 relationship with parameters on cardiac function in patients with T2D is warranted.

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

31 Hypothesis

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

37 Objectives

38 The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by
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40 ⁸²Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study, the
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42 effect of empagliflozin on key hemodynamic parameters will be measured during right heart catheterization at
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44 rest and during exercise. Key secondary outcomes include changes in cardiac echocardiographic evaluations of
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46 systolic and diastolic functions, functional capacity by accelerometry, and changes in glucose metabolism,
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48 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 outcomes²⁴. 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

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

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

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10 After 3 to 6 weeks of treatment, the participants will visit the trial site for blood tests and a physical
11 examination (V_2). Participant status and compliance as well as adverse events will be assessed at V_2 , V_3 and
12 during two phone calls (T_1 and T_2).
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16 17 **^{82}Rb -PET**

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

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

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18 The following echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular
19 ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial
20 volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric
21 relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-
22 chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a')
23 velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal
24 speckle tracking) will be assessed via 2D echocardiography. The longitudinal tissue velocity of the LV will
25 be assessed by averaging myocardial velocities and displacement of the mitral annular position in the
26 septal, lateral, inferior, posterior and anterior wall of the LV. Diastolic function will be assessed
27 according to EAE/ASE recommendations.
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38 **Cr-51 EDTA**

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

47 A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the
48 accelerometer continuously for 7 days, except for bathing and swimming.
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52 **Ambulatory blood pressure**

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4 24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM
5 Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every
6 hour during the night.
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10 11 **Adipose tissue biopsy**

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

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

32 Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.
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37 **Heart rate variability (HRV)**

38 Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be
39 measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and
40 while performing the Valsalva manoeuvre.
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46 **Pulse wave analysis (PWA)**

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

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4 Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA
5 scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software
6 provides a novel method for differentiating between visceral and subcutaneous abdominal fat.
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10 11 **Urine and blood samples, fasting**

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

26 27 **Primary outcome measurement**

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29 The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow
30 reserve MFR assessed by ^{82}Rb -PET
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32 **Key sub-study measurement**

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34 The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts
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37 **Secondary outcome measurements**

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39 The effect of empagliflozin compared with placebo on the following:

- 40 - Global left and right heart function
- 41 - Renal function by Cr51-EDTA plasma clearance
- 42 - Cardiac adipose tissue volume
- 43 - PCWP at peak exercise, corrected to body weight
- 44 - Plasma levels of NT-proBNP, MR-proANP, MR-proADM, GAL-3, hsTNT, GDF-15, PIGF, sFlt-1, FFA,
45 adiponectin, leptin, TNF- α , IL-6, MCP-1, MAC-1, COLL-A1 and FGF-21.
- 46 - Daily activity levels
- 47 - Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
- 48 - Ambulatory systolic and diastolic blood pressures
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- 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 \pm SD, and non-parametric statistics or appropriate log transformation will be

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4 performed if an assumption of normality is not met. After log transformation, the variable will be further tested
5 for normality distribution as indicated. A two-tailed p value of less than 0.05 will be considered statistically
6 significant.
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9 Comparisons between the treatment groups will be performed by an unpaired two sample t -test, Mann-
10 Whitney test or χ^2 - test as appropriate.
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12 13 Data management

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

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

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33 *Pharmaceutical Form:* Tablet for oral use

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

36 Intake of the tablet can be done at any time during the day; however, it is recommended that the time of
37 intake be consistent from day to day.
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40 *Side effects:* Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or
41 sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis,
42 vulvovaginitis
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45 *Shipping and packing:* All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

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47 *Randomization:* Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The
48 randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization
49 numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned
50 consecutively, and patients will retain these numbers following randomization.
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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 ⁸²Rb-PET measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.

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Competing interests:

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REFERENCES

1. Turner R. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998;352(9131):837-853. doi:10.1016/S0140-6736(98)07019-6
2. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus. *N Engl J Med*. 2013;369(14):1317-1326. doi:10.1056/NEJMoa1307684
3. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*. 2015;373(3):232-242. doi:10.1056/NEJMoa1501352
4. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *N Engl J Med*. 2015;373(23):2247-2257. doi:10.1056/NEJMoa1509225
5. White WB, Cannon CP, Heller SR, et al. Alogliptin after Acute Coronary Syndrome in Patients with Type 2 Diabetes. *N Engl J Med*. 2013;369(14):1327-1335. doi:10.1056/NEJMoa1305889
6. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med*. 2015;373(22):2117-2128. doi:10.1056/NEJMoa1504720
7. Guthrie R. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *Postgrad Med*. 2018;130(2):149-153. doi:10.1080/00325481.2018.1423852
8. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*. 2018;NEJMoa1812389. doi:10.1056/NEJMoa1812389
9. Cefalu WT, Kaul S, Gerstein HC, et al. Cardiovascular outcomes trials in type 2 diabetes: Where Do We Go From Here? Ref lections From a Diabetes Care Editors' Expert Forum. *Diabetes Care*. 2018;41(1):14-31. doi:10.2337/dci17-0057
10. Fitchett D, the E-REGO trial investigators, Zinman B, et al. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. *Eur*

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- Heart J.* 2016;37(19):1526-1534. doi:10.1093/eurheartj/ehv728
11. Abdul-Ghani M, Del Prato S, Chilton R, DeFronzo RA. SGLT2 Inhibitors and Cardiovascular Risk: Lessons Learned From the EMPA-REG OUTCOME Study. *Diabetes Care.* 2016;39(5):717-725. doi:10.2337/dc16-0041
 12. Avogaro A, Albiero M, Menegazzo L, De Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: The role of reparatory mechanisms. *Diabetes Care.* 2011;34(SUPPL. 2):285-290. doi:10.2337/dc11-s239
 13. Srivaratharajah K, Coutinho T, Dekemp R, et al. Reduced myocardial flow in heart failure patients with preserved ejection fraction. *Circ Hear Fail.* 2016;9(7). doi:10.1161/CIRCHEARTFAILURE.115.002562
 14. Murthy VL, Naya M, Foster CR, et al. Association between coronary vascular dysfunction and cardiac mortality in patients with and without diabetes mellitus. *Circulation.* 2012;126(15):1858-1868. doi:10.1161/CIRCULATIONAHA.112.120402
 15. Cortigiani L, Rigo F, Gherardi S, et al. Additional Prognostic Value of Coronary Flow Reserve in Diabetic and Nondiabetic Patients With Negative Dipyridamole Stress Echocardiography by Wall Motion Criteria. *J Am Coll Cardiol.* 2007;50(14):1354-1361. doi:10.1016/j.jacc.2007.06.027
 16. Lin B, Koibuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol.* 2014;13(1). doi:10.1186/s12933-014-0148-1
 17. Oelze M, Kröllner-Schön S, Welschof P, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. *PLoS One.* 2014;9(11):e112394. doi:10.1371/journal.pone.0112394
 18. Sattar N, McLaren J, Kristensen SL, Preiss D, McMurray JJ. SGLT2 Inhibition and cardiovascular events: why did EMPA-REG Outcomes surprise and what were the likely mechanisms? *Diabetologia.* 2016:1-7. doi:10.1007/s00125-016-3956-x
 19. Emil W, David K, A. BB, et al. Resting and exercise haemodynamics in relation to six-minute walk test in patients with heart failure and preserved ejection fraction. *Eur J Heart Fail.* 2017;20(4):715-722. doi:10.1002/ejhf.976
 20. Sharma S, Adroque J V, Golfman L, et al. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J.* 2004;18(14):1692-1700. doi:10.1096/fj.04-2263com
 21. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest.* 2014;124(2):499-508. doi:10.1172/JCI72227.uptake
 22. Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A “Thrifty Substrate” Hypothesis. *Diabetes Care.* 2016;(March):dc160330. doi:10.2337/dc16-0330
 23. Ridker PM. Clinician’s Guide to Reducing Inflammation to Reduce Atherothrombotic Risk. *J Am Coll Cardiol.* 2018;72(25):3320-3331. doi:10.1016/j.jacc.2018.06.082

- 1
2
3
4 24. Heise T, Seewaldt-Becker E, Macha S, et al. Safety, tolerability, pharmacokinetics and
5 pharmacodynamics following 4 weeks' treatment with empagliflozin once daily in patients with type 2
6 diabetes. *Diabetes, Obes Metab*. 2013;15(7):613-621. doi:10.1111/dom.12073
7
8
9 25. von Scholten BJ, Hasbak P, Christensen TE, et al. Cardiac 82Rb PET/CT for fast and non-invasive
10 assessment of microvascular function and structure in asymptomatic patients with type 2 diabetes.
11 *Diabetologia*. 2016;59(2):371-378. doi:10.1007/s00125-015-3799-x
12
13 26. Andersen MJ, Ersbøll M, Axelsson A, et al. Sildenafil and diastolic dysfunction after acute myocardial
14 infarction in patients with preserved ejection fraction: The sildenafil and diastolic dysfunction after
15 acute myocardial infarction (SIDAMI) trial. *Circulation*. 2013;127(11):1200-1208.
16 doi:10.1161/CIRCULATIONAHA.112.000056
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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 (>500 ms)
- ▶ 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 theophylline
- ▶ History of known or suspected bronchoconstrictive or bronchospastic lung disease (e.g., asthma)
- ▶ Not using safe contraception
- ▶ Pregnancy or desire hereof or breastfeeding.

Table 2 Overview of study visits

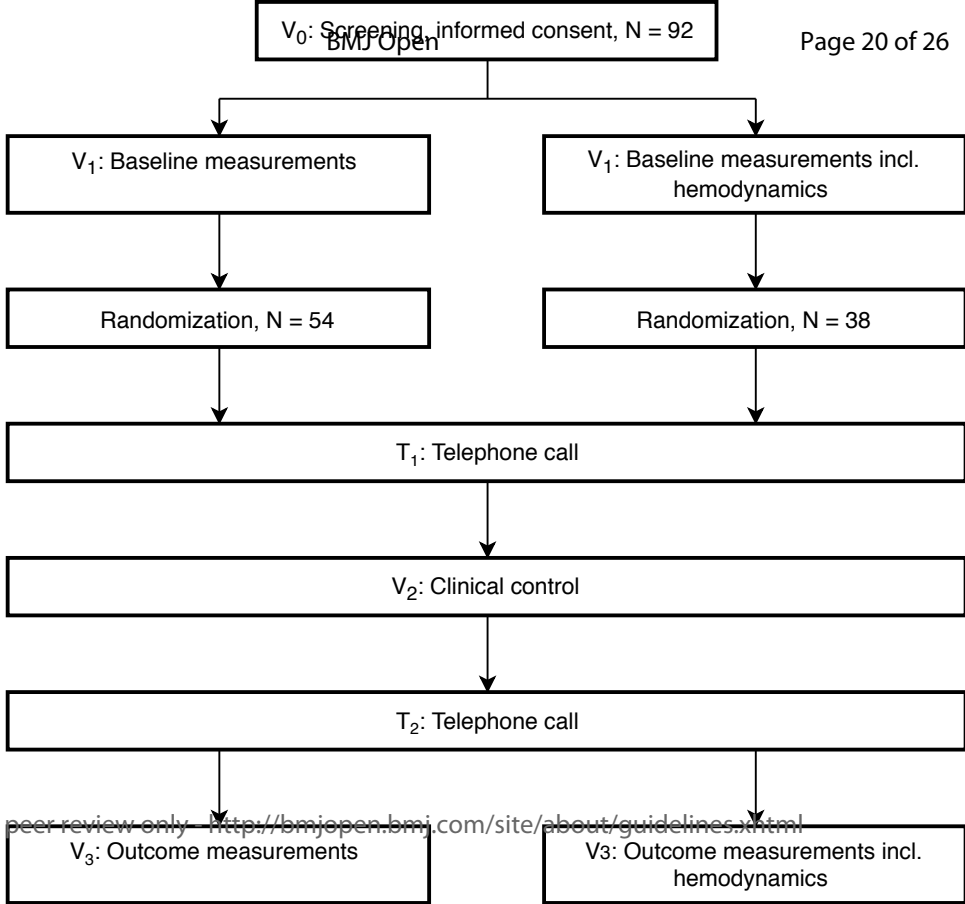
Visit	V ₀	V ₁	T ₁	V ₂	T ₂	V ₃
Time, weeks	-4±1	0±1	2±1	4±1	8±1	12±1
Inclusion/exclusion criteria	X	X				
Medical history	X					
Informed consent	X					
Blood samples, non-fasting	X			X		
Physical examination	X			X		
Adverse events		X	X	X	X	X
Endpoints, Main study						
⁸² Rb -PET (primary endpoint)		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

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Figure 1
Time-line of the study visits

Week -4±1
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7 Week 0±1
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11 Week 2±1
12
13
14
15 Week 4±1
16
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19 Week 8±1
20
21
22
23
24 Week 12±1
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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

1	sponsor contact			
2	information			
3				
4	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	See note
5	responsibilities:		collection, management, analysis, and interpretation of data; writing	3
6	sponsor and funder		of the report; and the decision to submit the report for publication,	
7			including whether they will have ultimate authority over any of these	
8			activities	
9				
10				
11				
12	Roles and	#5d	Composition, roles, and responsibilities of the coordinating centre,	n/a
13	responsibilities:		steering committee, endpoint adjudication committee, data	
14	committees		management team, and other individuals or groups overseeing the	
15			trial, if applicable (see Item 21a for data monitoring committee)	
16				
17				
18				
19	Background and	#6a	Description of research question and justification for undertaking the	2-4
20	rationale		trial, including summary of relevant studies (published and	
21			unpublished) examining benefits and harms for each intervention	
22				
23				
24	Background and	#6b	Explanation for choice of comparators	See note
25	rationale: choice of			4
26	comparators			
27				
28				
29	Objectives	#7	Specific objectives or hypotheses	4
30				
31				
32	Trial design	#8	Description of trial design including type of trial (eg, parallel group,	4
33			crossover, factorial, single group), allocation ratio, and framework	
34			(eg, superiority, equivalence, non-inferiority, exploratory)	
35				
36				
37	Study setting	#9	Description of study settings (eg, community clinic, academic	4
38			hospital) and list of countries where data will be collected. Reference	
39			to where list of study sites can be obtained	
40				
41				
42	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable,	14
43			eligibility criteria for study centres and individuals who will perform	
44			the interventions (eg, surgeons, psychotherapists)	
45				
46				
47				
48	Interventions:	#11a	Interventions for each group with sufficient detail to allow	4-5
49	description		replication, including how and when they will be administered	
50				
51				
52	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a	See note
53	modifications		given trial participant (eg, drug dose change in response to harms,	5
54			participant request, or improving / worsening disease)	
55				
56				
57	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any	See note
58	adherence		procedures for monitoring adherence (eg, drug tablet return;	6
59				
60				

		laboratory tests)	
1			
2			
3	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or
4	concomitant care		prohibited during the trial
5			
6	Outcomes	#12	Primary, secondary, and other outcomes, including the specific
7			measurement variable (eg, systolic blood pressure), analysis metric
8			(eg, change from baseline, final value, time to event), method of
9			aggregation (eg, median, proportion), and time point for each
10			outcome. Explanation of the clinical relevance of chosen efficacy
11			and harm outcomes is strongly recommended
12			
13			
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15			
16	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins
17			and washouts), assessments, and visits for participants. A schematic
18			diagram is highly recommended (see Figure)
19			
20			
21	Sample size	#14	Estimated number of participants needed to achieve study objectives
22			and how it was determined, including clinical and statistical
23			assumptions supporting any sample size calculations
24			
25			
26	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach
27			target sample size
28			
29			
30	Allocation: sequence	#16a	Method of generating the allocation sequence (eg, computer-
31	generation		generated random numbers), and list of any factors for stratification.
32			To reduce predictability of a random sequence, details of any
33			planned restriction (eg, blocking) should be provided in a separate
34			document that is unavailable to those who enrol participants or
35			assign interventions
36			
37			
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39			
40	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
41	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
42	mechanism		describing any steps to conceal the sequence until interventions are
43			assigned
44			
45			
46			
47	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
48	implementation		participants, and who will assign participants to interventions
49			
50			
51	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
52			participants, care providers, outcome assessors, data analysts), and
53			how
54			
55			
56	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible, and
57	emergency		procedure for revealing a participant's allocated intervention during
58			
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1	unblinding		the trial	
2				
3	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other	9
4			trial data, including any related processes to promote data quality	
5			(eg, duplicate measurements, training of assessors) and a description	
6			of study instruments (eg, questionnaires, laboratory tests) along with	
7			their reliability and validity, if known. Reference to where data	
8			collection forms can be found, if not in the protocol	
9				
10				
11				
12	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,	See note
13	retention		including list of any outcome data to be collected for participants	7
14			who discontinue or deviate from intervention protocols	
15				
16				
17	Data management	#19	Plans for data entry, coding, security, and storage, including any	9
18			related processes to promote data quality (eg, double data entry;	
19			range checks for data values). Reference to where details of data	
20			management procedures can be found, if not in the protocol	
21				
22				
23				
24	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes.	9
25			Reference to where other details of the statistical analysis plan can	
26			be found, if not in the protocol	
27				
28				
29				
30	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	9
31	analyses		analyses)	
32				
33				
34	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-adherence	9
35	population and		(eg, as randomised analysis), and any statistical methods to handle	
36	missing data		missing data (eg, multiple imputation)	
37				
38				
39	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of its	See note
40	formal committee		role and reporting structure; statement of whether it is independent	8
41			from the sponsor and competing interests; and reference to where	
42			further details about its charter can be found, if not in the protocol.	
43			Alternatively, an explanation of why a DMC is not needed	
44				
45				
46				
47	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	n/a
48	interim analysis		including who will have access to these interim results and make the	
49			final decision to terminate the trial	
50				
51				
52	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and	5
53			spontaneously reported adverse events and other unintended effects	
54			of trial interventions or trial conduct	
55				
56				
57				
58	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10
59				
60				

1			whether the process will be independent from investigators and the	
2			sponsor	
3				
4	Research ethics	#24	Plans for seeking research ethics committee / institutional review	See note
5	approval		board (REC / IRB) approval	9
6				
7				
8	Protocol amendments	#25	Plans for communicating important protocol modifications (eg,	See note
9			changes to eligibility criteria, outcomes, analyses) to relevant parties	10
10			(eg, investigators, REC / IRBs, trial participants, trial registries,	
11			journals, regulators)	
12				
13				
14	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial	4,5,11
15			participants or authorised surrogates, and how (see Item 32)	
16				
17				
18	Consent or assent:	#26b	Additional consent provisions for collection and use of participant	n/a
19	ancillary studies		data and biological specimens in ancillary studies, if applicable	
20				
21				
22	Confidentiality	#27	How personal information about potential and enrolled participants	9
23			will be collected, shared, and maintained in order to protect	
24			confidentiality before, during, and after the trial	
25				
26				
27	Declaration of	#28	Financial and other competing interests for principal investigators	11
28	interests		for the overall trial and each study site	
29				
30				
31	Data access	#29	Statement of who will have access to the final trial dataset, and	See note
32			disclosure of contractual agreements that limit such access for	11
33			investigators	
34				
35				
36	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and for	n/a
37	trial care		compensation to those who suffer harm from trial participation	(none)
38				
39				
40	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial results to	10
41	trial results		participants, healthcare professionals, the public, and other relevant	
42			groups (eg, via publication, reporting in results databases, or other	
43			data sharing arrangements), including any publication restrictions	
44				
45				
46				
47	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	See note
48	authorship		professional writers	12
49				
50				
51	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	10
52	reproducible research		participant-level dataset, and statistical code	
53				
54				
55	Informed consent	#32	Model consent form and other related documentation given to	See note
56	materials		participants and authorised surrogates	13
57				
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1 Biological specimens #33 Plans for collection, laboratory evaluation, and storage of biological n/a
2 specimens for genetic or molecular analysis in the current trial and
3 for future use in ancillary studies, if applicable
4
5
6

7 Author notes

- 9 1. n/a (not used)
- 11 2. 1 (The corresponding author is Sponsor-Investigator)
- 13 3. 1,11 (see above)
- 15 4. n/a (short, low risk trial)
- 17 5. n/a (no specific criteria)
- 19 6. n/a (no strategies)
- 21 7. n/a (no plans)
- 23 8. n/a (short, low risk study)
- 25 9. 2 (already approved)
- 27 10. n/a (no further modifications are expected)
- 29 11. 10 (public access)
- 31 12. n/a (standard guideline (Vancouver), no professional writers)
- 33 13. n/a (attached in seperate file)

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37 3.0. This checklist was completed on 11. January 2019 using <https://www.goodreports.org/>, a tool made by the
39 [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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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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Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Diabetic nephropathy & vascular disease < DIABETES & ENDOCRINOLOGY, RADIOLOGY, CARDIOLOGY, Cardiovascular imaging < RADIOLOGY & IMAGING

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Manuscripts

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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Mesh terms: Diabetes Mellitus, Type 2; Sodium-Glucose Transporter 2 Inhibitors, Cardiovascular Diseases, Myocardial Perfusion Imaging, Cardiac Catheterization

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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-

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

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

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

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

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

19 ARTICLE SUMMARY

20 Strengths and limitations of this study:

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

26 INTRODUCTION

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

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

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4 1 trials for new T2D drugs. Several such trials have been completed or are ongoing. The trials have primarily
5 2 examined the safety of dipeptidyl peptidase 4 (DPP-IV) inhibitors, glucagon-like peptide-1 receptor agonists
6 3 (GLP-1 RA) and sodium–glucose cotransporter 2 (SGLT2) inhibitors.^{2–8} The DPP-IV inhibitor trial SAVOR-TIMI
7 4 indicated an increase in the secondary endpoint, hospitalization due to HF with the active comparator, while
8 5 another DPP-IV trial, TECOS, as well as the newly published CARMELINA did not demonstrate an increased risk
9 6 with the active drug. The GLP-1 RA trials LEADER and SUSTAIN although with a reduction in the primary MACE
10 7 endpoint were neutral with regards to risk of HF. Most of the SGLT-2 trials have demonstrated CV benefits⁹ via
11 8 a reduction of the primary endpoint MACE. In the EMPA-REG outcome study, the SGLT-2 inhibitor empagliflozin
12 9 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
13 10 high-risk T2D population.^{6,10}

11 11 The novel evidence from the SGLT-2 inhibitor trials was surprising, because the treatments only had a modest
12 12 effect on classical CV risk factors. Indeed, the mechanism behind their profound effect on CV death and risk of
13 13 HF remains unknown, although several hypotheses have been proposed. An effect reducing the atherosclerotic
14 14 burden is considered unlikely, because the risk of CV events was reduced within weeks of treatment¹¹. Thus,
15 15 the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is
16 16 considered to play a significant role in T2D-related CVD¹² as well as in HF with preserved ejection fraction.¹³
17 17 Furthermore, coronary vascular dysfunction, particularly decreased vasodilator capacity, is a significant
18 18 predictor for CV death in patients with T2D.^{14,15} Notably, T2D patients with preserved coronary vasodilation
19 19 had the same low risk of CVD as subjects without diabetes¹⁴. Experimental studies have shown that SGLT-2
20 20 inhibitor treatment significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary
21 21 arterial fibrosis and improves vasodilatation in diabetic rodents.^{16,17} Thus, part of the underlying effect of
22 22 empagliflozin in EMPA-REG could be driven by improvement in myocardial microcirculation.

23 23 A complementary hypothesis has been suggested to explain the early beneficial effects on HF
24 24 hospitalization found in the EMPA-REG trial.¹⁸ Inhibition of SGLT-2 is associated with increased diuresis, thus
25 25 the sodium- and volume-reducing effect could favour cardiac loading conditions in patients with T2D with
26 26 subclinical dysfunctions of the heart. The proposed effects on central hemodynamics, especially right and left
27 27 heart filling pressures and cardiac output, can be measured directly by right heart catheterization. Pulmonary
28 28 capillary wedge pressure (PCWP), a measure of left heart filling pressure, has previously been shown to be
29 29 associated with functional capacity among patients with HF.¹⁹ Therefore, investigating the effect of SGLT-2
30 30 inhibitor on central hemodynamics during rest and exercise may contribute vital knowledge on the

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4 1 mechanisms behind the markedly reduced risk of admission for HF demonstrated in EMPA-REG and DECLAIRE
5
6 2 TIMI ^{6,8}.

7 3
8 4 The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR)
9
10 5 and adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating
11 6 ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac
12 7 function.²⁰ Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral
13 8 glucose uptake²¹ through a reduction in glucose toxicity. SGLT-2 inhibitors have been shown to increase the
14 9 glucagon-insulin ratio, presumably due to the decrease in glucose stimulus.²² Moreover they induce a small
15 10 increase in plasma ketone bodies, which some have proposed to be a preferred fuel source for the
16 11 myocardium.²³ However, others have shown that SGLT-2 inhibitors increase ATP production in mouse model
17 12 hearts, without increasing ketone oxidation.²⁴ Markers of inflammation have been associated with the risk of
18 13 vascular events, independently of traditional risk factors and an effect on markers of inflammation status such
19 14 as IL-6 and CRP should also be considered.²⁵ Therefore, further knowledge on the effects of SGLT-2 inhibitors
20 15 on metabolic parameters, such as biomarkers of inflammation, adipose tissue function and the relationship
21 16 with parameters on cardiac function in patients with T2D is warranted.

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31 17 In summary, SGLT2 inhibitors have been shown to reduce CVD in high-risk T2D patients, with several
32 18 explanatory hypotheses being suggested that remain to be tested in clinical trials.

33 34 35 19 Hypothesis

36 20 Treatment with empagliflozin for 13 weeks improves the myocardial flow reserve (MFR) in patients with T2D
37 21 and high cardiovascular risk. The duration of 13 weeks was chosen, as the EMPA-REG study showed a clear
38 22 effect on the primary outcomes at this timepoint.

39 40 41 42 23 Objectives

43 24 The primary objective of the current trial is to evaluate the effect of empagliflozin on MFR as measured by
44 25 ⁸²Rb-PET compared to placebo in patients with T2D and CVD or additional CV risk factors. In a sub study, the
45 26 effect of empagliflozin on key hemodynamic parameters will be measured during right heart catheterization at
46 27 rest and during exercise. Key secondary outcomes include changes in cardiac echocardiographic evaluations of
47 28 systolic and diastolic functions, functional capacity by accelerometry, and changes in glucose metabolism,
48 29 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 $\mu\text{g}/\text{kg}/\text{min}$ 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

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4 1 In a substudy, 38 participants randomly selected from the primary study population will perform a graded
5 2 exercise test until maximal exertion using a supine ergometer with simultaneous invasive hemodynamic
6 3 measurements. Using local anaesthesia, A Swan-Ganz catheter will be placed into the pulmonary artery
7 4 via the right internal jugular vein. The following hemodynamic variables will be assessed: pulmonary
8 5 capillary wedge pressure (PCWP), cardiac output (CO) using thermodilution, central venous pressure (CVP
9 6 and pulmonary artery pressure (PAP). Following measurements at rest, participants will be transferred to
10 7 a supine bicycle ergometer, and the measurements will be repeated (leg-raise situation). The participant
11 8 will be instructed to pedal at 60 rpm and the workload is incrementally increased at steps of 10watts.
12 9 Measurements during exercise will be obtained at 25 watts and during peak exercise. Blood sampled from
13 10 the pulmonary vein will be obtained at rest, 25 watts and at peak exercise for analyses of lactate, oxygen
14 11 saturation and other blood gas variables.
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13 **Echocardiography**

14 The following echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular
15 15 ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial
16 16 volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric
17 17 relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-
18 18 chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a')
19 19 velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal
20 20 speckle tracking) will be assessed via 2D echocardiography. The longitudinal tissue velocity of the LV will
21 21 be assessed by averaging myocardial velocities and displacement of the mitral annular position in the
22 22 septal, lateral, inferior, posterior and anterior wall of the LV. Diastolic function will be assessed
23 23 according to EAE/ASE recommendations.
24 24

25 **Cr-51 EDTA**

26 26 The Cr-51 EDTA method will be used to measure glomerular filtration rate (GFR). A single intravenous
27 27 administration of 3.7MBq of Cr-51 EDTA is given. A venous sample is drawn four hours after
28 28 administration.
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1 **Accelerometry**

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

4 **Ambulatory blood pressure**

5 24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM
6 Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every
7 hour during the night.

8 **Adipose tissue biopsy**

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

15 **Oral Glucose Tolerance Test (OGTT)**

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

19 **Questionnaires**

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

21 **Heart rate variability (HRV)**

22 Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be
23 measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and
24 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

- 1 - 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.
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- 3 - Daily activity levels
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- 5 - Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
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- 7 - Ambulatory systolic and diastolic blood pressures
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- 9 - Quality of life assessed by the Minnesota Living with Heart Failure and the EuroQol EQ-5D-5L questionnaires
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- 11 - Plasma beta-hydroxy butyrate levels
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- 13 - Urine albumin/creatinine ratio
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- 15 - 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

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4 1 global MBF from the baseline to week 13 between the treatment and control groups. The primary analysis will
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6 2 be based on the ITT population. The primary outcome measure will be analysed using ANCOVA with treatment
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8 3 as a factor and the baseline value as a covariate. The model will include the prespecified covariates age and
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10 4 gender. Missing data will be estimated using the maximum likelihood method. Normally distributed variables
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12 5 will be presented as mean \pm SD, and non-parametric statistics or appropriate log transformation will be
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14 6 performed if an assumption of normality is not met. After log transformation, the variable will be further tested
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16 7 for normality distribution as indicated. A two-tailed p value of less than 0.05 will be considered statistically
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18 8 significant.

19 9 Comparisons between the treatment groups will be performed by an unpaired two sample t -test, Mann-
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21 10 Whitney test or χ^2 - test as appropriate.

22 11 Data management

23 12 Source data will be recorded in the patient record or in case report forms (CRF). The requirements for entering
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25 13 source data directly into the CRF are that the data are obtained directly from the patient either by clinical
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27 14 assessment, interview or point-of-care systems with no printout function and that no more reliable forms of
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29 15 data capture are available. Medical history, height and weight are examples of such data.

30 16 A CRF will be constructed for data capture. Data will be stored in coded form for 5 years according to
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32 17 recommendations from the Danish Data Protection Agency; thereafter, data will be transferred to the Danish
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34 18 Data Archives.

39 20 Study medication

40 21 *Name:* Jardiance® (empagliflozin) or a visually identical matching placebo

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42 22 *Pharmaceutical Form:* Tablet for oral use

43 23 *Pharmacological Dosage:* Jardiance® or a placebo will be introduced at a dose of 25 mg/day.

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45 24 Intake of the tablet can be done at any time during the day; however, it is recommended that the time of
46
47 25 intake be consistent from day to day.

48 26 *Side effects:* Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or
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50 27 sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis,
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52 28 vulvovaginitis

53 29 *Shipping and packing:* All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

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4 1 *Randomization:* Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The
5 2 randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization
6 3 numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned
7 4 consecutively, and patients will retain these numbers following randomization.
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10 5 Concomitant medication

11 6 Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant
12 7 medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving
13 8 optimal T2D therapy, and during the trial, T2D and CV risk or disease will be managed according to the best
14 9 available evidence.
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19 10 ETHICS AND DISSEMINATION

20 11 The study, which will be conducted in accordance with the latest revision of the Helsinki Declaration, EU
21 12 directive on GCP and ICH-GCP guidelines, has been approved by the Regional Scientific Ethics Committee, the
22 13 Danish Medicines Agency and the Danish Data Protection Agency. The study is registered at clinicaltrials.gov
23 14 and monitored by the GCP unit at Bispebjerg University Hospital. The results of the project will be submitted to
24 15 international peer-reviewed journals regardless of their outcome, and the data will be made available to the
25 16 public via EudraCT and www.clinicaltrials.gov. Furthermore, the results will be presented at conferences as
26 17 abstracts and posters.
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56 36 Author Contributions:

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4 1 CK, MS, FG, AK, PH, JF, BZ, SI and MJ conceived the study and participated in its design, planning and
5 2 coordination. CK, MS, MW, PG, PR, NB and MJ are responsible for the inclusion and examination of patients at
6 3 Herlev-Gentofte and Rigshospitalet University Hospital. AK and PH are responsible for the ⁸²Rb-PET
7 4 measurements and FG, EW for the hemodynamics experiments at Rigshospitalet University Hospital.
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17 9 ClinicalTrials.gov Identifier: NCT03151343, EudraCT number: 2016-003743-10
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27 15 REFERENCES

- 28
29 16 1. Turner R. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional
30 17 treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*.
31 18 1998;352(9131):837-853. doi:10.1016/S0140-6736(98)07019-6
 - 32
33 19 2. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and Cardiovascular Outcomes in Patients with Type
34 20 2 Diabetes Mellitus. *N Engl J Med*. 2013;369(14):1317-1326. doi:10.1056/NEJMoa1307684
 - 35
36 21 3. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2
37 22 Diabetes. *N Engl J Med*. 2015;373(3):232-242. doi:10.1056/NEJMoa1501352
 - 38
39 23 4. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary
40 24 Syndrome. *N Engl J Med*. 2015;373(23):2247-2257. doi:10.1056/NEJMoa1509225
 - 41
42 25 5. White WB, Cannon CP, Heller SR, et al. Alogliptin after Acute Coronary Syndrome in Patients with Type 2
43 26 Diabetes. *N Engl J Med*. 2013;369(14):1327-1335. doi:10.1056/NEJMoa1305889
 - 44
45 27 6. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2
46 28 Diabetes. *N Engl J Med*. 2015;373(22):2117-2128. doi:10.1056/NEJMoa1504720
 - 47
48 29 7. Guthrie R. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *Postgrad Med*.
49 30 2018;130(2):149-153. doi:10.1080/00325481.2018.1423852
 - 50
51 31 8. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N*
52 32 *Engl J Med*. 2018;NEJMoa1812389. doi:10.1056/NEJMoa1812389
- 53
54
55
56
57
58
59
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- 1
2
3
4 1 9. Cefalu WT, Kaul S, Gerstein HC, et al. Cardiovascular outcomes trials in type 2 diabetes: Where Do We
5 2 Go From Here? Ref lections From a Diabetes Care Editors' Expert Forum. *Diabetes Care*. 2018;41(1):14-
6 3 31. doi:10.2337/dci17-0057
7
8 4 10. Fitchett D, the E-REGO trial investigators, Zinman B, et al. Heart failure outcomes with empagliflozin in
9 5 patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. *Eur*
10 6 *Heart J*. 2016;37(19):1526-1534. doi:10.1093/eurheartj/ehv728
11
12 7 11. Abdul-Ghani M, Del Prato S, Chilton R, DeFronzo RA. SGLT2 Inhibitors and Cardiovascular Risk: Lessons
13 8 Learned From the EMPA-REG OUTCOME Study. *Diabetes Care*. 2016;39(5):717-725. doi:10.2337/dc16-
14 9 0041
15
16 10 12. Avogaro A, Albiero M, Menegazzo L, De Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes:
17 11 The role of reparatory mechanisms. *Diabetes Care*. 2011;34(SUPPL. 2):285-290. doi:10.2337/dc11-s239
18
19 12 13. Srivaratharajah K, Coutinho T, Dekemp R, et al. Reduced myocardial flow in heart failure patients with
20 13 preserved ejection fraction. *Circ Hear Fail*. 2016;9(7). doi:10.1161/CIRCHEARTFAILURE.115.002562
21
22 14 14. Murthy VL, Naya M, Foster CR, et al. Association between coronary vascular dysfunction and cardiac
23 15 mortality in patients with and without diabetes mellitus. *Circulation*. 2012;126(15):1858-1868.
24 16 doi:10.1161/CIRCULATIONAHA.112.120402
25
26 17 15. Cortigiani L, Rigo F, Gherardi S, et al. Additional Prognostic Value of Coronary Flow Reserve in Diabetic
27 18 and Nondiabetic Patients With Negative Diprydamole Stress Echocardiography by Wall Motion Criteria.
28 19 *J Am Coll Cardiol*. 2007;50(14):1354-1361. doi:10.1016/j.jacc.2007.06.027
29
30 20 16. Lin B, Koibuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2
31 21 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice.
32 22 *Cardiovasc Diabetol*. 2014;13(1). doi:10.1186/s12933-014-0148-1
33
34 23 17. Oelze M, Kröller-Schön S, Welschof P, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin
35 24 improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering
36 25 with oxidative stress and glucotoxicity. *PLoS One*. 2014;9(11):e112394.
37 26 doi:10.1371/journal.pone.0112394
38
39 27 18. Sattar N, McLaren J, Kristensen SL, Preiss D, McMurray JJ. SGLT2 Inhibition and cardiovascular events:
40 28 why did EMPA-REG Outcomes surprise and what were the likely mechanisms? *Diabetologia*. 2016:1-7.
41 29 doi:10.1007/s00125-016-3956-x
42
43 30 19. Emil W, David K, A. BB, et al. Resting and exercise haemodynamics in relation to six-minute walk test in
44 31 patients with heart failure and preserved ejection fraction. *Eur J Heart Fail*. 2017;20(4):715-722.
45 32 doi:10.1002/ejhf.976
46
47 33 20. Sharma S, Adroque J V, Golfman L, et al. Intramyocardial lipid accumulation in the failing human heart
48 34 resembles the lipotoxic rat heart. *FASEB J*. 2004;18(14):1692-1700. doi:10.1096/fj.04-2263com
49
50 35 21. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2
51 36 inhibition in type 2 diabetic patients. *J Clin Invest*. 2014;124(2):499-508. doi:10.1172/JCI72227.uptake
52
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51
52
53
54
55
56
57
58
59
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- 1 22. Ferrannini E. Sodium-Glucose Co-transporters and Their Inhibition: Clinical Physiology. *Cell Metab.* 2017;26(1):27-38. doi:10.1016/j.cmet.2017.04.011
- 2
- 3
- 4 23. Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A “Thrifty Substrate” Hypothesis. *Diabetes Care.* 2016;(March):dc160330. doi:10.2337/dc16-0330
- 5
- 6
- 7 24. Verma S, Rawat S, Ho KL, et al. Empagliflozin Increases Cardiac Energy Production in Diabetes. *JACC Basic to Transl Sci.* 2018. doi:10.1016/j.jacbts.2018.07.006
- 8
- 9
- 10 25. Ridker PM. Clinician’s Guide to Reducing Inflammation to Reduce Atherothrombotic Risk. *J Am Coll Cardiol.* 2018;72(25):3320-3331. doi:10.1016/j.jacc.2018.06.082
- 11
- 12
- 13 26. Heise T, Seewaldt-Becker E, Macha S, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics following 4 weeks’ treatment with empagliflozin once daily in patients with type 2 diabetes. *Diabetes, Obes Metab.* 2013;15(7):613-621. doi:10.1111/dom.12073
- 14
- 15
- 16 27. von Scholten BJ, Hasbak P, Christensen TE, et al. Cardiac 82Rb PET/CT for fast and non-invasive assessment of microvascular function and structure in asymptomatic patients with type 2 diabetes. *Diabetologia.* 2016;59(2):371-378. doi:10.1007/s00125-015-3799-x
- 17
- 18
- 19 28. Andersen MJ, Ersbøll M, Axelsson A, et al. Sildenafil and diastolic dysfunction after acute myocardial infarction in patients with preserved ejection fraction: The sildenafil and diastolic dysfunction after acute myocardial infarction (SIDAMI) trial. *Circulation.* 2013;127(11):1200-1208. doi:10.1161/CIRCULATIONAHA.112.000056
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- 21
- 22
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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 (>500 ms)
- ▶ 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 theophylline
- ▶ History of known or suspected bronchoconstrictive or bronchospastic lung disease (e.g., asthma)
- ▶ Not using safe contraception
- ▶ Pregnancy or desire hereof or breastfeeding.

Table 2 Overview of study visits

Visit	V ₀	V ₁	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

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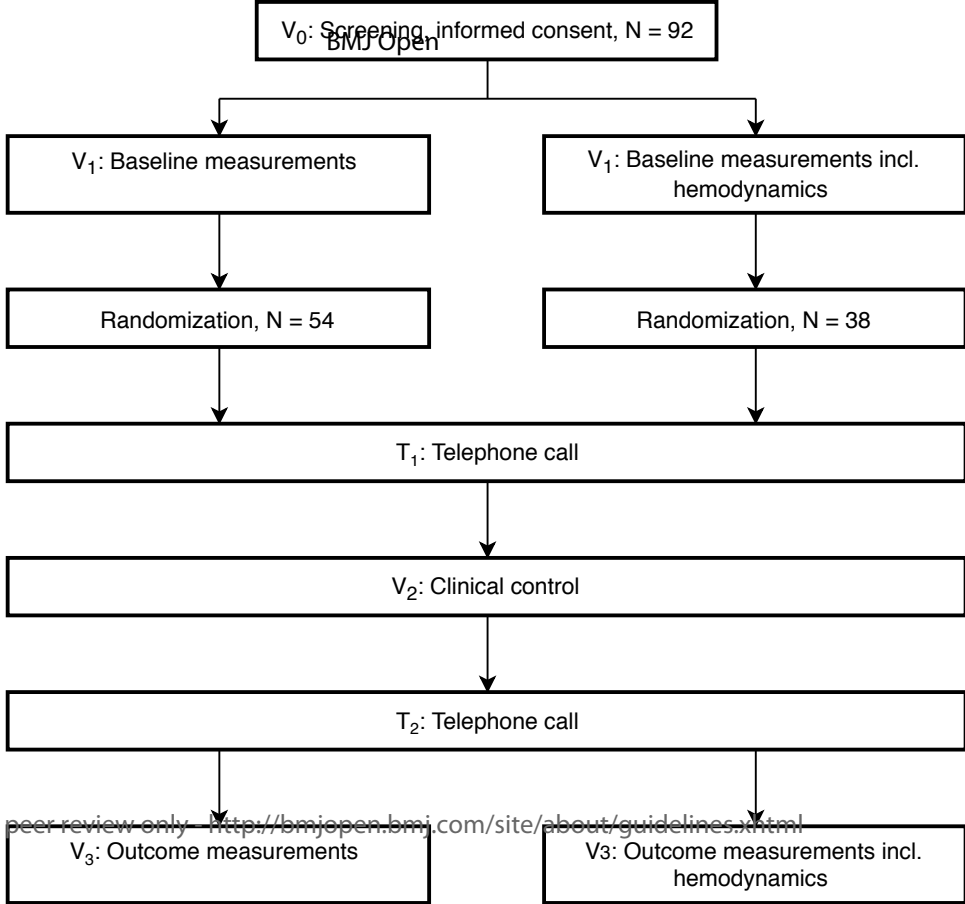
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For peer review only

Figure 1
Time-line of the study visits

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7 Week 0±1
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11 Week 2±1
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14
15 Week 4±1
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18
19 Week 8±1
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23
24 Week 12±1
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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

1	sponsor contact			
2	information			
3				
4	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	See note
5	responsibilities:		collection, management, analysis, and interpretation of data; writing	3
6	sponsor and funder		of the report; and the decision to submit the report for publication,	
7			including whether they will have ultimate authority over any of these	
8			activities	
9				
10				
11				
12	Roles and	#5d	Composition, roles, and responsibilities of the coordinating centre,	n/a
13	responsibilities:		steering committee, endpoint adjudication committee, data	
14	committees		management team, and other individuals or groups overseeing the	
15			trial, if applicable (see Item 21a for data monitoring committee)	
16				
17				
18				
19	Background and	#6a	Description of research question and justification for undertaking the	2-4
20	rationale		trial, including summary of relevant studies (published and	
21			unpublished) examining benefits and harms for each intervention	
22				
23				
24	Background and	#6b	Explanation for choice of comparators	See note
25	rationale: choice of			4
26	comparators			
27				
28				
29	Objectives	#7	Specific objectives or hypotheses	4
30				
31				
32	Trial design	#8	Description of trial design including type of trial (eg, parallel group,	4
33			crossover, factorial, single group), allocation ratio, and framework	
34			(eg, superiority, equivalence, non-inferiority, exploratory)	
35				
36				
37	Study setting	#9	Description of study settings (eg, community clinic, academic	4
38			hospital) and list of countries where data will be collected. Reference	
39			to where list of study sites can be obtained	
40				
41				
42	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable,	14
43			eligibility criteria for study centres and individuals who will perform	
44			the interventions (eg, surgeons, psychotherapists)	
45				
46				
47				
48	Interventions:	#11a	Interventions for each group with sufficient detail to allow	4-5
49	description		replication, including how and when they will be administered	
50				
51				
52	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a	See note
53	modifications		given trial participant (eg, drug dose change in response to harms,	5
54			participant request, or improving / worsening disease)	
55				
56				
57	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any	See note
58	adherence		procedures for monitoring adherence (eg, drug tablet return;	6
59				
60				

		laboratory tests)	
1			
2			
3	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or
4	concomitant care		prohibited during the trial
5			10
6	Outcomes	#12	Primary, secondary, and other outcomes, including the specific
7			measurement variable (eg, systolic blood pressure), analysis metric
8			(eg, change from baseline, final value, time to event), method of
9			aggregation (eg, median, proportion), and time point for each
10			outcome. Explanation of the clinical relevance of chosen efficacy
11			and harm outcomes is strongly recommended
12			8
13			
14			
15			
16	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins
17			and washouts), assessments, and visits for participants. A schematic
18			diagram is highly recommended (see Figure)
19			15
20			
21	Sample size	#14	Estimated number of participants needed to achieve study objectives
22			and how it was determined, including clinical and statistical
23			assumptions supporting any sample size calculations
24			9
25			
26	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach
27			target sample size
28			4
29			
30	Allocation: sequence	#16a	Method of generating the allocation sequence (eg, computer-
31	generation		generated random numbers), and list of any factors for stratification.
32			To reduce predictability of a random sequence, details of any
33			planned restriction (eg, blocking) should be provided in a separate
34			document that is unavailable to those who enrol participants or
35			assign interventions
36			4-5
37			
38			
39			
40	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
41	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
42	mechanism		describing any steps to conceal the sequence until interventions are
43			assigned
44			4-5
45			
46			
47	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
48	implementation		participants, and who will assign participants to interventions
49			4-5, 11
50			
51	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
52			participants, care providers, outcome assessors, data analysts), and
53			how
54			4
55			
56	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible, and
57	emergency		procedure for revealing a participant's allocated intervention during
58			4-5
59			
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1	unblinding		the trial	
2				
3	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other	9
4			trial data, including any related processes to promote data quality	
5			(eg, duplicate measurements, training of assessors) and a description	
6			of study instruments (eg, questionnaires, laboratory tests) along with	
7			their reliability and validity, if known. Reference to where data	
8			collection forms can be found, if not in the protocol	
9				
10				
11				
12	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,	See note
13	retention		including list of any outcome data to be collected for participants	7
14			who discontinue or deviate from intervention protocols	
15				
16				
17	Data management	#19	Plans for data entry, coding, security, and storage, including any	9
18			related processes to promote data quality (eg, double data entry;	
19			range checks for data values). Reference to where details of data	
20			management procedures can be found, if not in the protocol	
21				
22				
23				
24	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes.	9
25			Reference to where other details of the statistical analysis plan can	
26			be found, if not in the protocol	
27				
28				
29				
30	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	9
31	analyses		analyses)	
32				
33				
34	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-adherence	9
35	population and		(eg, as randomised analysis), and any statistical methods to handle	
36	missing data		missing data (eg, multiple imputation)	
37				
38				
39	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of its	See note
40	formal committee		role and reporting structure; statement of whether it is independent	8
41			from the sponsor and competing interests; and reference to where	
42			further details about its charter can be found, if not in the protocol.	
43			Alternatively, an explanation of why a DMC is not needed	
44				
45				
46				
47	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	n/a
48	interim analysis		including who will have access to these interim results and make the	
49			final decision to terminate the trial	
50				
51				
52				
53	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and	5
54			spontaneously reported adverse events and other unintended effects	
55			of trial interventions or trial conduct	
56				
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58	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10
59				
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		whether the process will be independent from investigators and the sponsor	
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3			
4	Research ethics approval	#24 Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	See note 9
5			
6			
7			
8	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
9			
10			
11			
12			
13			
14	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
15			
16			
17			
18	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
19			
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22	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
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27	Declaration of interests	#28 Financial and other competing interests for principal investigators for the overall trial and each study site	11
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31	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
32			
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36	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)
37			
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40	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
41			
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47	Dissemination policy: authorship	#31b Authorship eligibility guidelines and any intended use of professional writers	See note 12
48			
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51	Dissemination policy: reproducible research	#31c Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	10
52			
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55	Informed consent materials	#32 Model consent form and other related documentation given to participants and authorised surrogates	See note 13
56			
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1 Biological specimens #33 Plans for collection, laboratory evaluation, and storage of biological n/a
2 specimens for genetic or molecular analysis in the current trial and
3 for future use in ancillary studies, if applicable
4
5
6

7 Author notes

- 9 1. n/a (not used)
- 11 2. 1 (The corresponding author is Sponsor-Investigator)
- 13 3. 1,11 (see above)
- 15 4. n/a (short, low risk trial)
- 17 5. n/a (no specific criteria)
- 19 6. n/a (no strategies)
- 21 7. n/a (no plans)
- 23 8. n/a (short, low risk study)
- 25 9. 2 (already approved)
- 27 10. n/a (no further modifications are expected)
- 29 11. 10 (public access)
- 31 12. n/a (standard guideline (Vancouver), no professional writers)
- 33 13. n/a (attached in seperate file)

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37 3.0. This checklist was completed on 11. January 2019 using <https://www.goodreports.org/>, a tool made by the
39 [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-029098.R3
Article Type:	Protocol
Date Submitted by the Author:	29-Oct-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, Department of medicine Gæde, Peter; Slagelse Sygehus Rossing, Peter; Steno Diabetes Center AS Faber, J; Herlev Hospital, Medicine Inzucchi, Silvio; Yale School 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, RADIOLOGY, CARDIOLOGY, Cardiovascular imaging < RADIOLOGY & IMAGING

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Manuscripts

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6 1 Design of a randomized controlled trial of the effects of empagliflozin on
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9 2 myocardial perfusion, function and metabolism in type 2 diabetes patients
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12 3 at high cardiovascular risk (The SIMPLE trial)
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17 5 Mikkel Jürgens¹, Morten Schou^{2,3}, Philip Hasbak⁴, Andreas Kjær⁴, Emil Wolsk⁵, Bo Zerahn⁶,

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21 6 Mikkel Wiberg¹, Niels Brandt⁷, Peter Gæde⁸, Peter Rossing^{3,9}, Jens Faber^{1,3}, Silvio E.

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24 7 Inzucchi¹⁰, Finn Gustafsson^{4,9}, Caroline Kistorp^{3,7}
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40 16 **Word count: 3147**

41
42 17 **Mesh terms: Diabetes Mellitus, Type 2; Sodium-Glucose Transporter 2 Inhibitors, Cardiovascular Diseases,**
43 18 **Myocardial Perfusion Imaging, Cardiac Catheterization**

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47 20 **Copenhagen; +45 3545 9642; caroline.michaela.kistorp@regionh.dk**
48
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50 21 **ABSTRACT**
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4 1 **Introduction:** A diagnosis of type 2 diabetes (T2D) more than doubles the risk of cardiovascular disease
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7 2 (CVD), with heart failure (HF) being one of the most common complications with a severe prognosis. The
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10 3 landmark EMPA-REG study demonstrated that treatment with the sodium glucose cotransporter-2 (SGLT-
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12 4 2) inhibitor empagliflozin rapidly and significantly reduces CVD mortality and admission rates for HF.
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14
15 5 However, the mechanisms behind this reduction in clinical events are unknown.

16
17 6 The current study was designed to investigate the effects of the SGLT-2 inhibitor empagliflozin on
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20 7 myocardial perfusion and function in patients with T2D and high CVD risk.

21
22 8 **Methods and analysis:** In this investigator-initiated, randomized, double-blind controlled clinical trial, 92
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25 9 patients with T2D and established CVD or high CVD risk, will be randomized to treatment with empagliflozin
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28 10 25 mg or a matching placebo for 13 weeks. The primary outcome measure is change in myocardial flow
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31 11 reserve measured quantitatively by Rubidium-82 position emission tomography (⁸²Rb-PET). In a substudy,
32
33 12 invasive hemodynamics at rest and during exercise will be measured at baseline and following the
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36 13 intervention, using right heart catheterization.

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38 14 **Ethics and dissemination:** The study protocol (v7, 02/08/2018) has been approved by the Ethics Committee
39
40
41 15 of the Capital Region, Danish Data Protection Board and the Danish Medicines Agency, and it will be
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43
44 16 monitored according to the Good Clinical Practice (GCP) regulations from the International Conference on
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46 17 Harmonization. The results be submitted to international peer-reviewed journals and be presented at
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49 18 conferences. The data will be made available to the public via EudraCT and www.clinicaltrials.gov.

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51 19 **Trial Registration:** ClinicalTrials.gov Identifier: NCT03151343, EudraCT number: 2016-003743-10
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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 benefits⁹ 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 treatment¹¹. Thus, the cardioprotective effect might be functional rather than anatomical. Impaired myocardial microcirculation is considered to play a significant role in T2D-related CVD¹² 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

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4 1 low risk of CVD as subjects without diabetes¹⁴. Experimental studies have shown that SGLT-2 inhibitor treatment
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6 2 significantly ameliorates coronary arterial and aortic (media) thickening, pericoronary arterial fibrosis and
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8 3 improves vasodilatation in diabetic rodents.^{16,17} Thus, part of the underlying effect of empagliflozin in EMPA-REG
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10 4 could be driven by improvement in myocardial microcirculation.

11 5 A complementary hypothesis has been suggested to explain the early beneficial effects on HF
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13 6 hospitalization found in the EMPA-REG trial.¹⁸ Inhibition of SGLT-2 is associated with increased diuresis, thus the
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15 7 sodium- and volume-reducing effect could favour cardiac loading conditions in patients with T2D with subclinical
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17 8 dysfunctions of the heart. The proposed effects on central hemodynamics, especially right and left heart filling
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19 9 pressures and cardiac output, can be measured directly by right heart catheterization. Pulmonary capillary wedge
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21 10 pressure (PCWP), a measure of left heart filling pressure, has previously been shown to be associated with
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23 11 functional capacity among patients with HF.¹⁹ Therefore, investigating the effect of SGLT-2 inhibitor on central
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25 12 hemodynamics during rest and exercise may contribute vital knowledge on the mechanisms behind the markedly
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27 13 reduced risk of admission for HF demonstrated in EMPA-REG and DECLAIRe TIMI ^{6,8}.

28 14 The pathogenesis of myocardial dysfunction in T2D has been linked with insulin resistance (IR) and
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30 15 adipose tissue dysfunction, with an increased supply of free fatty acids (FFA) and adipocytokines mediating
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32 16 ectopic fat storage and thus increased intra-myocardial lipid accumulation, with decremental impact on cardiac
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34 17 function.²⁰ Increased glycosuria with SGLT-2 inhibitors could improve IR by indirectly increasing peripheral
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36 18 glucose uptake²¹ through a reduction in glucose toxicity. SGLT-2 inhibitors have been shown to increase the
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38 19 glucagon-insulin ratio, presumably due to the decrease in glucose stimulus.²² Moreover they induce a small
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40 20 increase in plasma ketone bodies, which some have proposed to be a preferred fuel source for the myocardium.²³
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42 21 However, others have shown that SGLT-2 inhibitors increase ATP production in mouse model hearts, without
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44 22 increasing ketone oxidation.²⁴ Markers of inflammation have been associated with the risk of vascular events,
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46 23 independently of traditional risk factors and an effect on markers of inflammation status such as IL-6 and CRP
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48 24 should also be considered.²⁵ Therefore, further knowledge on the effects of SGLT-2 inhibitors on metabolic
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50 25 parameters, such as biomarkers of inflammation, adipose tissue function and the relationship with parameters
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52 26 on cardiac function in patients with T2D is warranted.

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60 27 In summary, SGLT2 inhibitors have been shown to reduce CVD in high-risk T2D patients, with several explanatory
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62 28 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 catheterization 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

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4 1 participants. A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicated a dose-response
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6 2 effect on metabolic outcomes such as urinary glucose excretion, fasting plasma glucose and mean daily plasma
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8 3 glucose, with higher drug doses resulting in lower glucose levels and increased glucose excretion.²⁶. In
9
10 4 accordance with the EMPA-REG study, no dose-escalation will be performed. The trial medication will be
11
12 5 randomized in computer-generated 1:1 blocks of 10 by the study pharmacy (Glostrup Pharmacy, Copenhagen,
13
14 6 Denmark). Participants will receive randomization numbers and the corresponding medication containers
15
16 7 sequentially. In case of medical emergency, participants can be unblinded individually. The study medicine will
17
18 8 be suspended if the participant becomes pregnant or withdraws consent. The investigators may suspend the
19
20 9 medication for safety reasons.
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22
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24 11 Patient and public involvement

25 12 The study was designed without involvement from patients or the public. The patients will be offered access to
26
27 13 the results of the study, when these become available.
28
29 14

32 15 Trial visits and procedures

33 16 Visits

35 17 A schematic overview of the trial visits is presented in Table 2 and as a flowchart in Figure 1. At the screening
36
37 18 visit (V_0), informed consent will be obtained, and patients will be assessed for eligibility based on medical history,
38
39 19 including diabetes duration and comorbidities, physical examinations and blood samples. Ineligible participants
40
41 20 will be counted as screen failures, and the reason for screen failure will be recorded.

42 21 Outcome-related procedures are performed at the baseline (V_1) and after 13 weeks of treatment (V_3). The trial
43
44 22 medication will be dispensed when all the procedures pertaining to V_1 have been completed.

45 23 After 3 to 6 weeks of treatment, the participants will visit the trial site for blood tests and a physical examination
46
47 24 (V_2). Participant status and compliance as well as adverse events will be assessed at V_2 , V_3 and during two phone
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49 25 calls (T_1 and T_2).
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52 27 ^{82}Rb -PET

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4 1 Myocardial perfusion and myocardial flow reserve (MFR) will be measured using cardiac ^{82}Rb -PET, which allows
5
6 2 for flow quantification in absolute terms. To determine the MFR, measurements will be performed at rest and
7
8 3 during adenosine-induced stress. A standard clinical protocol will be used. Participants will be scanned in the
9
10 4 supine position using a Siemens Biograph mCT/PET 128-slice scanner (Siemens Medical Solutions, Knoxville,
11
12 5 USA). Low-dose non-contrast CT will be acquired for attenuation correction as well as measurement of cardiac
13
14 6 adipose tissue volume. Approximately 1100 MBq of ^{82}Rb will be obtained from a CardioGen-82 Sr-82/Rb-82
15
16 7 generator (Bracco Diagnostics, Inc., Princeton, New Jersey, USA) and intravenously infused with a constant
17
18 8 flowrate of 50 mL/min. List-mode 3D data acquisition will begin with the tracer infusion and continue for 7
19
20 9 minutes. Static and ECG-gated images will be reconstructed with a 2.5-minute delay to allow ^{82}Rb to clear from
21
22 10 the blood pool. Maximal hyperaemia will be induced with adenosine infused at 140 $\mu\text{g}/\text{kg}/\text{min}$ for 6 minutes.
23
24 11 After 2.5 minutes of adenosine infusion, intravenous ^{82}Rb infusion and list-mode acquisition will follow the
25
26 12 same protocol as for rest. Myocardial blood perfusion quantification (in mL/min/g) will be performed using
27
28 13 Cedars-Sinai QGS+QPS 2015.6 software (Cedars-Sinai Medical Center), which is based on a single-compartment
29
30 14 model for Rb-82 tracer kinetics.
31

32 **Hemodynamics sub-study**

33 17 In a substudy, 38 participants randomly selected from the primary study population will perform a graded
34
35 18 exercise test until maximal exertion using a supine ergometer with simultaneous invasive hemodynamic
36
37 19 measurements. Using local anaesthesia, A Swan-Ganz catheter will be placed into the pulmonary artery via
38
39 20 the right internal jugular vein. The following hemodynamic variables will be assessed: pulmonary capillary
40
41 21 wedge pressure (PCWP), cardiac output (CO) using thermodilution, central venous pressure (CVP and
42
43 22 pulmonary artery pressure (PAP). Following measurements at rest, participants will be transferred to a
44
45 23 supine bicycle ergometer, and the measurements will be repeated (leg-raise situation). The participant will
46
47 24 be instructed to pedal at 60 rpm and the workload is incrementally increased at steps of 10watts.
48
49 25 Measurements during exercise will be obtained at 25 watts and during peak exercise. Blood sampled from
50
51 26 the pulmonary vein will be obtained at rest, 25 watts and at peak exercise for analyses of lactate, oxygen
52
53 27 saturation and other blood gas variables.
54

55 **Echocardiography**

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4 1 The following echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular
5 2 ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial
6 3 volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric
7 4 relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-
8 5 chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a') velocities
9 6 during the ejection period. Strain measures of LV (radial, circumferential and longitudinal speckle tracking)
10 7 will be assessed via 2D echocardiography. The longitudinal tissue velocity of the LV will be assessed by
11 8 averaging myocardial velocities and displacement of the mitral annular position in the septal, lateral,
12 9 inferior, posterior and anterior wall of the LV. Diastolic function will be assessed according to EAE/ASE
13 10 recommendations.
14 11

12 **Cr-51 EDTA**

13 The Cr-51 EDTA method will be used to measure glomerular filtration rate (GFR). A single intravenous
14 administration of 3.7MBq of Cr-51 EDTA is given. A venous sample is drawn four hours after administration.
15

16 **Accelerometry**

17 A patient-worn accelerometer will be used to assess daily activity level. The patient will wear the
18 accelerometer continuously for 7 days, except for bathing and swimming.
19

20 **Ambulatory blood pressure**

21 24-hour ambulatory blood pressures will be obtained using a Mobil-O-Graph New Generation 24h ABPM
22 Classic (Siemens, Germany) set to measure blood pressure every 20 minutes during the daytime and every
23 hour during the night.
24

25 **Adipose tissue biopsy**

26 Biopsies will be obtained from the abdominal subcutaneous tissue lateral to the umbilicus during a fasting
27 state using the Bergstrom needle technique. Following dissection, the biopsies are washed and snap frozen
28 in liquid nitrogen and stored at -80°C. Total mRNA will be extracted from adipose tissue with commercially
29 available lipid tissue kits to measure the mRNA expression of biomarkers of interest. Protein expression will
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3
4 1 be analysed using Western blots. Quantification of inflammatory cells will be performed according to
5
6 2 validated histological procedures. Fibrosis levels will be measured using the Sirius Red stain.
7
8 3

9 4 **Oral Glucose Tolerance Test (OGTT)**

10
11 5 The fasting participant will drink a solution of 75g of glucose. Blood will be drawn at time points 0, 30, 60
12
13 6 and 120 minutes, and measurements of plasma glucose, C-peptide and plasma insulin will be taken. Frozen
14
15 7 aliquots for measurement of glucagon will be kept for a later sub-study.
16
17 8

18 9 **Questionnaires**

19
20 10 Participants will complete the EQ-5D-5L and the Minnesota Living with Heart Failure questionnaires.
21
22 11

23 12 **Heart rate variability (HRV)**

24
25 13 Heart rate variability will be measured using the Vagus™ handheld device. Heart rate response will be
26
27 14 measured at rest, while rising from a lying position to standing upright, during deep in- and expiration and
28
29 15 while performing the Valsalva manoeuvre.
30
31 16

32 17 **Pulse wave analysis (PWA)**

33
34 18 Arterial stiffness will be measured using PWA. The procedure will be performed using a SphygmoCor device
35
36 19 (version 7.0, Atcor Medical, Sydney, Australia) at the right radial artery.
37
38 20

39 21 **Body composition**

40
41 22 Body composition will be measured by Dual-energy X-ray absorptiometry (DXA) (Hologic Discovery DXA
42
43 23 scanner, Hologic Inc., Bedford, USA) based on fat free, fat and bone mass. Supplementary software provides
44
45 24 a novel method for differentiating between visceral and subcutaneous abdominal fat.
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47 25

48 26 **Urine and blood samples, fasting**

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4 1 Blood will be drawn in the fasting state, and patients will provide a sample of first morning urine. Standard
5
6 2 blood analyses will be performed immediately after sampling. Urine samples for measurement of
7
8 3 albumin/creatinine ratio and blood samples for specialized biomarkers will be stored at -80°C until batch
9
10 4 analysis can be performed. Blood analysis will include inflammatory biomarkers such as interleukin-6 (IL-6)
11
12 5 and tumor necrosis factor- α (TNF- α), cardiospecific biomarkers such as N-terminal pro-B-type natriuretic
13
14 6 peptide (NT-proBNP) and high-sensitivity troponin T (hs-TNT), as well as markers of adipocyte function,
15
16 7 including adiponectin and leptin.

17 8 ENDPOINTS

19 9 **Primary outcome measurement**

20
21 10 The effect of empagliflozin 25 mg once daily for 13 weeks, compared with placebo on the myocardial flow reserve
22
23 11 MFR assessed by ^{82}Rb -PET

24 12 **Key sub-study measurement**

25
26 13 The effect of empagliflozin compared with placebo on PCWP at a workload of 25 watts
27
28 14

29 15 **Secondary outcome measurements**

30
31 16 The effect of empagliflozin compared with placebo on the following:

- 32
33 17 - Global left and right heart function
- 34 18 - Renal function by Cr51-EDTA plasma clearance
- 35 19 - Cardiac adipose tissue volume
- 36 20 - PCWP at peak exercise, corrected to body weight
- 37 21 - Plasma levels of NT-proBNP, MR-proANP, MR-proADM, GAL-3, hsTNT, GDF-15, PIGF, sFlt-1, FFA,
38 22 adiponectin, leptin, TNF- α , IL-6, MCP-1, MAC-1, COLL-A1, endothelin-1 and FGF-21.
- 39
40 23 - Daily activity levels
- 41 24 - Body fat distribution and visceral vs. subcutaneous abdominal fat using DXA
- 42 25 - Ambulatory systolic and diastolic blood pressures
- 43 26 - Quality of life assessed by the Minnesota Living with Heart Failure and the EuroQol EQ-5D-5L
44 27 questionnaires
- 45
46 28 - Plasma beta-hydroxy butyrate levels
- 47 29 - Urine albumin/creatinine ratio
- 48 30 - Adipose tissue fibrosis, mRNA and protein expression of TNF- α , adiponectin, IL-6, COL1-A1, MAC-1, FGF-
49 31 21, and monocyte chemoattractant protein 1 (MCP1)

1 STATISTICAL ANALYSIS

2 SAMPLE SIZE

3 Primary endpoint

The

4 primary endpoint is change in MFR. In a previous study on a comparable population, the SD of measurements
5 of MFR by ^{82}Rb PET was 0.8. Assuming a clinically relevant difference between the treatment and placebo
6 groups after 6 months of 0.5 in MFR,²⁷ a sample size of 41:41 (empagliflozin: placebo) can be detected with
7 80% power and a two-sided significance level of 5%. In all, 92 patients will be randomized to allow for a 10%
8 drop-out rate from the intention-to-treat (ITT) population.

9 Sub-study key endpoint

10 Based on recent experiments in a comparable study population,²⁸ a difference of 5 mmHg in PCWP during
11 exercise was considered clinically significant. The SD of measurements of PCWP was 5 mmHg; thus, a sample
12 size of 16:16 is required with a power of 80% and a two-sided significance level of 5% to detect a difference of
13 5 mmHg between groups. In total 38 patients will be included in the sub-study to allow for a 15% drop-out
14 rate.

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

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4 1 Comparisons between the treatment groups will be performed by an unpaired two sample *t*-test, Mann-Whitney
5
6 2 test or χ^2 - test as appropriate.
7

9 3 Data management

10 4 Source data will be recorded in the patient record or in case report forms (CRF). The requirements for entering
11
12 5 source data directly into the CRF are that the data are obtained directly from the patient either by clinical
13
14 6 assessment, interview or point-of-care systems with no printout function and that no more reliable forms of
15
16 7 data capture are available. Medical history, height and weight are examples of such data.

17 8 A CRF will be constructed for data capture. Data will be stored in coded form for 5 years according to
18
19 9 recommendations from the Danish Data Protection Agency; thereafter, data will be transferred to the Danish
20
21 10 Data Archives.
22

26 12 Study medication

27
28 13 *Name:* Jardiance® (empagliflozin) or a visually identical matching placebo

29
30 14 *Pharmaceutical Form:* Tablet for oral use

31
32 15 *Pharmacological Dosage:* Jardiance® or a placebo will be introduced at a dose of 25 mg/day.

33 16 Intake of the tablet can be done at any time during the day; however, it is recommended that the time of
34
35 17 intake be consistent from day to day.

36 18 *Side effects:* Very common side effects (> 10%): Hypoglycaemia (when taken in conjunction with insulin or
37
38 19 sulfonylureas); Common side effects (1–10%): Skin itching, balanitis, frequent urination, vaginal candidiasis,
39
40 20 vulvovaginitis

41 21 *Shipping and packing:* All trial products will be delivered, packed and labelled by Glostrup Pharmacy.

42
43 22 *Randomization:* Electronic randomization in blocks of 10 will be provided by Glostrup Pharmacy. The
44
45 23 randomization list will be stored in a locked cabinet. The patients will be assigned consecutive randomization
46
47 24 numbers. Prior to randomization, the patients will be identified by patient numbers which will be assigned
48
49 25 consecutively, and patients will retain these numbers following randomization.

50 26 Concomitant medication

51 27 Treatment with herbal medicines is not allowed, but otherwise, there are no restrictions on concomitant
52
53 28 medication apart from SGLT2-inhibitors. Before enrolment, participants will confirm that they are receiving
54

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

3 ETHICS AND DISSEMINATION

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

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29 **Author Contributions:**

30 CK, MS, FG, AK, PH, JF, BZ, SI and MJ conceived the study and participated in its design, planning and
31 coordination. CK, MS, MW, PG, PR, NB and MJ are responsible for the inclusion and examination of patients at
32 Herlev-Gentofte and Rigshospitalet University Hospital. AK and PH are responsible for the ⁸²Rb-PET
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19 10 REFERENCES

- 22 11 1. Turner R. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional
23 12 treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*.
24 13 1998;352(9131):837-853. doi:10.1016/S0140-6736(98)07019-6
- 26 14 2. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and Cardiovascular Outcomes in Patients with Type
27 15 2 Diabetes Mellitus. *N Engl J Med*. 2013;369(14):1317-1326. doi:10.1056/NEJMoa1307684
- 29 16 3. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2
30 17 Diabetes. *N Engl J Med*. 2015;373(3):232-242. doi:10.1056/NEJMoa1501352
- 32 18 4. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary
33 19 Syndrome. *N Engl J Med*. 2015;373(23):2247-2257. doi:10.1056/NEJMoa1509225
- 35 20 5. White WB, Cannon CP, Heller SR, et al. Alogliptin after Acute Coronary Syndrome in Patients with Type 2
36 21 Diabetes. *N Engl J Med*. 2013;369(14):1327-1335. doi:10.1056/NEJMoa1305889
- 38 22 6. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2
39 23 Diabetes. *N Engl J Med*. 2015;373(22):2117-2128. doi:10.1056/NEJMoa1504720
- 41 24 7. Guthrie R. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *Postgrad Med*.
42 25 2018;130(2):149-153. doi:10.1080/00325481.2018.1423852
- 44 26 8. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N*
45 27 *Engl J Med*. 2018;NEJMoa1812389. doi:10.1056/NEJMoa1812389
- 47 28 9. Cefalu WT, Kaul S, Gerstein HC, et al. Cardiovascular outcomes trials in type 2 diabetes: Where Do We
48 29 Go From Here? Ref lections From a Diabetes Care Editors' Expert Forum. *Diabetes Care*. 2018;41(1):14-
49 30 31. doi:10.2337/dci17-0057
- 51 31 10. Fitchett D, the E-REGO trial investigators, Zinman B, et al. Heart failure outcomes with empagliflozin in
52 32 patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. *Eur*
53 33 *Heart J*. 2016;37(19):1526-1534. doi:10.1093/eurheartj/ehv728

- 1
2
3
4 1 11. Abdul-Ghani M, Del Prato S, Chilton R, DeFronzo RA. SGLT2 Inhibitors and Cardiovascular Risk: Lessons
5 2 Learned From the EMPA-REG OUTCOME Study. *Diabetes Care*. 2016;39(5):717-725. doi:10.2337/dc16-
6 3 0041
7
8 4 12. Avogaro A, Albiero M, Menegazzo L, De Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes:
9 5 The role of reparatory mechanisms. *Diabetes Care*. 2011;34(SUPPL. 2):285-290. doi:10.2337/dc11-s239
10
11 6 13. Srivaratharajah K, Coutinho T, Dekemp R, et al. Reduced myocardial flow in heart failure patients with
12 7 preserved ejection fraction. *Circ Hear Fail*. 2016;9(7). doi:10.1161/CIRCHEARTFAILURE.115.002562
13
14 8 14. Murthy VL, Naya M, Foster CR, et al. Association between coronary vascular dysfunction and cardiac
15 9 mortality in patients with and without diabetes mellitus. *Circulation*. 2012;126(15):1858-1868.
16 10 doi:10.1161/CIRCULATIONAHA.112.120402
17
18 11 15. Cortigiani L, Rigo F, Gherardi S, et al. Additional Prognostic Value of Coronary Flow Reserve in Diabetic
19 12 and Nondiabetic Patients With Negative Dipyridamole Stress Echocardiography by Wall Motion Criteria.
20 13 *J Am Coll Cardiol*. 2007;50(14):1354-1361. doi:10.1016/j.jacc.2007.06.027
21
22 14 16. Lin B, Koibuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2
23 15 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice.
24 16 *Cardiovasc Diabetol*. 2014;13(1). doi:10.1186/s12933-014-0148-1
25
26 17 17. Oelze M, Kröller-Schön S, Welschof P, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin
27 18 improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering
28 19 with oxidative stress and glucotoxicity. *PLoS One*. 2014;9(11):e112394.
29 20 doi:10.1371/journal.pone.0112394
30
31 21 18. Sattar N, McLaren J, Kristensen SL, Preiss D, McMurray JJ. SGLT2 Inhibition and cardiovascular events:
32 22 why did EMPA-REG Outcomes surprise and what were the likely mechanisms? *Diabetologia*. 2016:1-7.
33 23 doi:10.1007/s00125-016-3956-x
34
35 24 19. Emil W, David K, A. BB, et al. Resting and exercise haemodynamics in relation to six-minute walk test in
36 25 patients with heart failure and preserved ejection fraction. *Eur J Heart Fail*. 2017;20(4):715-722.
37 26 doi:10.1002/ejhf.976
38
39 27 20. Sharma S, Adroque J V, Golfman L, et al. Intramyocardial lipid accumulation in the failing human heart
40 28 resembles the lipotoxic rat heart. *FASEB J*. 2004;18(14):1692-1700. doi:10.1096/fj.04-2263com
41
42 29 21. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2
43 30 inhibition in type 2 diabetic patients. *J Clin Invest*. 2014;124(2):499-508. doi:10.1172/JCI72227.uptake
44
45 31 22. Ferrannini E. Sodium-Glucose Co-transporters and Their Inhibition: Clinical Physiology. *Cell Metab*.
46 32 2017;26(1):27-38. doi:10.1016/j.cmet.2017.04.011
47
48 33 23. Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A “Thrifty Substrate”
49 34 Hypothesis. *Diabetes Care*. 2016;(March):dc160330. doi:10.2337/dc16-0330
50
51 35 24. Verma S, Rawat S, Ho KL, et al. Empagliflozin Increases Cardiac Energy Production in Diabetes. *JACC*
52 36 *Basic to Transl Sci*. 2018. doi:10.1016/j.jacbts.2018.07.006
53
54
55
56
57
58
59

- 1
2
3
4 1 25. Ridker PM. Clinician's Guide to Reducing Inflammation to Reduce Atherothrombotic Risk. *J Am Coll*
5 2 *Cardiol.* 2018;72(25):3320-3331. doi:10.1016/j.jacc.2018.06.082
6
7 3 26. Heise T, Seewaldt-Becker E, Macha S, et al. Safety, tolerability, pharmacokinetics and
8 4 pharmacodynamics following 4 weeks' treatment with empagliflozin once daily in patients with type 2
9 5 diabetes. *Diabetes, Obes Metab.* 2013;15(7):613-621. doi:10.1111/dom.12073
10
11 6 27. von Scholten BJ, Hasbak P, Christensen TE, et al. Cardiac 82Rb PET/CT for fast and non-invasive
12 7 assessment of microvascular function and structure in asymptomatic patients with type 2 diabetes.
13 8 *Diabetologia.* 2016;59(2):371-378. doi:10.1007/s00125-015-3799-x
14
15 9 28. Andersen MJ, Ersbøll M, Axelsson A, et al. Sildenafil and diastolic dysfunction after acute myocardial
16 10 infarction in patients with preserved ejection fraction: The sildenafil and diastolic dysfunction after
17 11 acute myocardial infarction (SIDAMI) trial. *Circulation.* 2013;127(11):1200-1208.
18 12 doi:10.1161/CIRCULATIONAHA.112.000056
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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 (>500 ms)
- ▶ 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	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

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For peer review only

Figure 1
Time-line of the study visits

Week -4±1

V₀: Screening, informed consent, N = 92
BMJ Open

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V₁: Baseline measurements

V₁: Baseline measurements incl. hemodynamics

Randomization, N = 54

Randomization, N = 38

Week 0±1

T₁: Telephone call

Week 2±1

V₂: Clinical control

Week 4±1

T₂: Telephone call

Week 8±1

V₃: Outcome measurements

V₃: Outcome measurements incl. hemodynamics

Week 12±1

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

1	sponsor contact			
2	information			
3				
4	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	See note
5	responsibilities:		collection, management, analysis, and interpretation of data; writing	3
6	sponsor and funder		of the report; and the decision to submit the report for publication,	
7			including whether they will have ultimate authority over any of these	
8			activities	
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11				
12	Roles and	#5d	Composition, roles, and responsibilities of the coordinating centre,	n/a
13	responsibilities:		steering committee, endpoint adjudication committee, data	
14	committees		management team, and other individuals or groups overseeing the	
15			trial, if applicable (see Item 21a for data monitoring committee)	
16				
17				
18				
19	Background and	#6a	Description of research question and justification for undertaking the	2-4
20	rationale		trial, including summary of relevant studies (published and	
21			unpublished) examining benefits and harms for each intervention	
22				
23				
24	Background and	#6b	Explanation for choice of comparators	See note
25	rationale: choice of			4
26	comparators			
27				
28				
29	Objectives	#7	Specific objectives or hypotheses	4
30				
31				
32	Trial design	#8	Description of trial design including type of trial (eg, parallel group,	4
33			crossover, factorial, single group), allocation ratio, and framework	
34			(eg, superiority, equivalence, non-inferiority, exploratory)	
35				
36				
37	Study setting	#9	Description of study settings (eg, community clinic, academic	4
38			hospital) and list of countries where data will be collected. Reference	
39			to where list of study sites can be obtained	
40				
41				
42	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable,	14
43			eligibility criteria for study centres and individuals who will perform	
44			the interventions (eg, surgeons, psychotherapists)	
45				
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47				
48	Interventions:	#11a	Interventions for each group with sufficient detail to allow	4-5
49	description		replication, including how and when they will be administered	
50				
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52	Interventions:	#11b	Criteria for discontinuing or modifying allocated interventions for a	See note
53	modifications		given trial participant (eg, drug dose change in response to harms,	5
54			participant request, or improving / worsening disease)	
55				
56				
57	Interventions:	#11c	Strategies to improve adherence to intervention protocols, and any	See note
58	adherence		procedures for monitoring adherence (eg, drug tablet return;	6
59				
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		laboratory tests)	
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3	Interventions:	#11d	Relevant concomitant care and interventions that are permitted or
4	concomitant care		prohibited during the trial
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6	Outcomes	#12	Primary, secondary, and other outcomes, including the specific
7			measurement variable (eg, systolic blood pressure), analysis metric
8			(eg, change from baseline, final value, time to event), method of
9			aggregation (eg, median, proportion), and time point for each
10			outcome. Explanation of the clinical relevance of chosen efficacy
11			and harm outcomes is strongly recommended
12			
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16	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins
17			and washouts), assessments, and visits for participants. A schematic
18			diagram is highly recommended (see Figure)
19			
20			
21	Sample size	#14	Estimated number of participants needed to achieve study objectives
22			and how it was determined, including clinical and statistical
23			assumptions supporting any sample size calculations
24			
25			
26	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach
27			target sample size
28			
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30	Allocation: sequence	#16a	Method of generating the allocation sequence (eg, computer-
31	generation		generated random numbers), and list of any factors for stratification.
32			To reduce predictability of a random sequence, details of any
33			planned restriction (eg, blocking) should be provided in a separate
34			document that is unavailable to those who enrol participants or
35			assign interventions
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40	Allocation	#16b	Mechanism of implementing the allocation sequence (eg, central
41	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
42	mechanism		describing any steps to conceal the sequence until interventions are
43			assigned
44			
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47	Allocation:	#16c	Who will generate the allocation sequence, who will enrol
48	implementation		participants, and who will assign participants to interventions
49			
50			
51	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial
52			participants, care providers, outcome assessors, data analysts), and
53			how
54			
55			
56	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible, and
57	emergency		procedure for revealing a participant's allocated intervention during
58			
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1	unblinding		the trial	
2				
3	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other	9
4			trial data, including any related processes to promote data quality	
5			(eg, duplicate measurements, training of assessors) and a description	
6			of study instruments (eg, questionnaires, laboratory tests) along with	
7			their reliability and validity, if known. Reference to where data	
8			collection forms can be found, if not in the protocol	
9				
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12	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,	See note
13	retention		including list of any outcome data to be collected for participants	7
14			who discontinue or deviate from intervention protocols	
15				
16				
17	Data management	#19	Plans for data entry, coding, security, and storage, including any	9
18			related processes to promote data quality (eg, double data entry;	
19			range checks for data values). Reference to where details of data	
20			management procedures can be found, if not in the protocol	
21				
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24	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes.	9
25			Reference to where other details of the statistical analysis plan can	
26			be found, if not in the protocol	
27				
28				
29				
30	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	9
31	analyses		analyses)	
32				
33				
34	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-adherence	9
35	population and		(eg, as randomised analysis), and any statistical methods to handle	
36	missing data		missing data (eg, multiple imputation)	
37				
38				
39	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of its	See note
40	formal committee		role and reporting structure; statement of whether it is independent	8
41			from the sponsor and competing interests; and reference to where	
42			further details about its charter can be found, if not in the protocol.	
43			Alternatively, an explanation of why a DMC is not needed	
44				
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47	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	n/a
48	interim analysis		including who will have access to these interim results and make the	
49			final decision to terminate the trial	
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52	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and	5
53			spontaneously reported adverse events and other unintended effects	
54			of trial interventions or trial conduct	
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58	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	10
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1			whether the process will be independent from investigators and the	
2			sponsor	
3				
4	Research ethics	#24	Plans for seeking research ethics committee / institutional review	See note
5	approval		board (REC / IRB) approval	9
6				
7				
8	Protocol amendments	#25	Plans for communicating important protocol modifications (eg,	See note
9			changes to eligibility criteria, outcomes, analyses) to relevant parties	10
10			(eg, investigators, REC / IRBs, trial participants, trial registries,	
11			journals, regulators)	
12				
13				
14	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial	4,5,11
15			participants or authorised surrogates, and how (see Item 32)	
16				
17				
18	Consent or assent:	#26b	Additional consent provisions for collection and use of participant	n/a
19	ancillary studies		data and biological specimens in ancillary studies, if applicable	
20				
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22	Confidentiality	#27	How personal information about potential and enrolled participants	9
23			will be collected, shared, and maintained in order to protect	
24			confidentiality before, during, and after the trial	
25				
26				
27	Declaration of	#28	Financial and other competing interests for principal investigators	11
28	interests		for the overall trial and each study site	
29				
30				
31	Data access	#29	Statement of who will have access to the final trial dataset, and	See note
32			disclosure of contractual agreements that limit such access for	11
33			investigators	
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36	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and for	n/a
37	trial care		compensation to those who suffer harm from trial participation	(none)
38				
39				
40	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial results to	10
41	trial results		participants, healthcare professionals, the public, and other relevant	
42			groups (eg, via publication, reporting in results databases, or other	
43			data sharing arrangements), including any publication restrictions	
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47	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	See note
48	authorship		professional writers	12
49				
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51	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	10
52	reproducible research		participant-level dataset, and statistical code	
53				
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55	Informed consent	#32	Model consent form and other related documentation given to	See note
56	materials		participants and authorised surrogates	13
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1 Biological specimens #33 Plans for collection, laboratory evaluation, and storage of biological n/a
2 specimens for genetic or molecular analysis in the current trial and
3 for future use in ancillary studies, if applicable
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7 Author notes

- 9 1. n/a (not used)
- 11 2. 1 (The corresponding author is Sponsor-Investigator)
- 13 3. 1,11 (see above)
- 15 4. n/a (short, low risk trial)
- 17 5. n/a (no specific criteria)
- 19 6. n/a (no strategies)
- 21 7. n/a (no plans)
- 23 8. n/a (short, low risk study)
- 25 9. 2 (already approved)
- 27 10. n/a (no further modifications are expected)
- 29 11. 10 (public access)
- 31 12. n/a (standard guideline (Vancouver), no professional writers)
- 33 13. n/a (attached in seperate file)

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37 3.0. This checklist was completed on 11. January 2019 using <https://www.goodreports.org/>, a tool made by the
39 [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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