PROTOCOL TITLE DiEtary Sodium Intake effects on ertugliflozin-induced changes in GFR, reNal oxygenation and systemic hemodynamics: the DESIGN study, a randomized, placebo-controlled, cross-over study with ertugliflozin in people with type 2 diabetes

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Short title	**DESIGN study**
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Coordinating investigator/project leader	**Dr. D.H. van Raalte** Department of Internal Medicine, Amsterdam UMC De Boelelaan 1117 1081 HV Amsterdam Room ZH 4A-58 TEL: +31-20-4440534 E-mail: d.vanraalte@amsterdamumc.nl
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	**Dr. D.H. van Raalte** Department of Internal Medicine, Amsterdam UMC De Boelelaan 1117 1081 HV Amsterdam Room ZH 4A-58 TEL: +31-20-4440534 E-mail: d.vanraalte@amsterdamumc.nl
Multicenter research: per site	**Dr. Petter Bjornstad** Department of Medicine, Division of Nephrology and Hypertension; Department of Pediatrics, Section Endocrinology and Diabetes. University of Colorado School of Medicine. 13123 16th Ave Box B265 Aurora, CO 80045, USA TEL: +1 720 777 4659 E-mail: Petter.Bjornstad@childrenscolorado.org

Sponsor (in Dutch: verrichter/opdrachtgever)	**Diabetes Center, Department of internal medicine, Amsterdam UMC, location VUMC**
Subsidising party	MSD, the proposal is an **investigator-initiated study**
Independent expert	**Dr. E. Peters** Department of Internal Medicine, Amsterdam UMC De Boelelaan 1117 1081 HV Amsterdam Room PK-1Z-108 TEL: +31-20-4440596 E-mail: e.peters@amsterdamumc.nl
Laboratory sites	**Department of Clinical Chemistry and Laboratory of Endocrinology Amsterdam UMC**
Pharmacy	**Trial pharmacy Amsterdam UMC**

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Prof. J. Prins **Head of department Internal Medicine**		
Dr. D.H. van Raalte **Principle Investigator**		

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: SGLT2 inhibitors such as ertugliflozin improve blood pressure and kidney outcomes in people living with diabetes through incompletely understood mechanisms, however, not all patients treated with SGLT2 inhibition have improved outcomes. Changes in kidney sodium handling is among the mechanisms by which SGLT2 inhibition may reduce blood pressure and drive beneficial kidney outcomes. This process is heavily dependent on daily sodium intake by patients receiving SGLT2 inhibitor treatment. In this study, the effect of daily sodium intake on SGLT2-inhibitor induced physiological effect is studied, including blood pressure regulation and kidney physiology.

Objective: Primary outcome: to investigate the modifying effects of WHO-recommended sodium intake (90 mmol per day) vs. high sodium intake (targeted at 250 mmol per day) on the effect of ertugliflozin 15 mg daily, versus placebo, on 24-hour blood pressure in overweight/obese adults with type 2 diabetes.

Study design: The study is a bi-center randomized, placebo-controlled, crossover intervention study. Total number of included participants is 34, which undergo 4 conditions in randomized order (randomization list generated by computer and supervised by pharmacy; blinded to investigator). While the treatment will be blinded for all participants, the sodium interventions are open-label.

Study population: 34 Adults living with type 2 diabetes. 18 - 85 years of age. Obese or overweight with BMI > 25 kg/m^2.

Intervention (if applicable): Cross-over design during 4 conditions. The 4 conditions are: 1) low-sodium diet; placebo; 2) low-sodium diet; ertugliflozin 15 once daily; 3) high-sodium diet; placebo; 4) high-sodium diet; ertugliflozin 15 mg once daily

Main study parameters/endpoints:

Primary endpoint is indicated above.

Secondary endpoint: To investigate the efficacy of ertugliflozin 15 mg daily, versus placebo, in overweight/obese adults with type 2 diabetes to reduce the hypertensive effects of a high-sodium diet (250 mmol per day) versus 24-h blood pressure measurement during participant's normal diet (170 mmol/per day) obtained at screening visit.

Exploratory endpoints: To investigate the modifying effects of WHO-recommended sodium intake (90 mmol per day) vs. high sodium intake (targeted at 250 mmol per day) on the effect of ertugliflozin 15 mg daily, versus placebo, on the following parameters in overweight/obese adults with type 2 diabetes on GFR by iohexol clearance, Hematocrit, Office blood pressure, Kidney oxygenation, Parameters of intra kidney hemodynamic function including ERPF by p-aminohippurate (PAH) clearance and renal vascular resistance, Body anthropometrics, Fasting plasma glucose, Albumin excretion rate (24 hour), 24-hr urinary glucose excretion, Urinary and plasma biomarkers.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

There is no clear health benefit for the study participants, except that participation will provide insight into their sodium intake and knowledge how to reduce sodium intake to WHO-recommended amounts, which may benefit their long-term health. The short-term treatment with SGLT2 inhibitor ertugliflozin will not provide lasting benefits. On the other hand, the drug has little side effects, especially during short-term treatment, which will be closely monitored. The most commonly observed side effect (usually after more prolonged treatment) involves genital mycotic infections, which can easily be treated with antifungal topicals. Glucose levels and blood pressure are lowered during treatment. There are no health risks to short-term changes in sodium diet. Potential changes in blood pressure during the high-sodium diet are closely monitored. The study procedures are safe. There are no risks with undergoing MRI procedures and the use of iohexol and PAH to measure kidney hemodynamic function bears no risk, with >1500 tests carried out in our department. Burden of participants includes 5 visits (including screening); 4 visits where an MRI scan (duration 30 min) is performed and where 2 intravenous catheters are placed for tracer infusion during 2,5 hours; 13 24-hr urine collections and 5 24-hr blood pressure collections. A total of maximally 500 mL blood will be withdrawn over the course of a number of months. The study team will mitigate travel for the participants by collecting 24-hr blood pressure and 24-urine samples at home.

INTRODUCTION AND RATIONALE

Diabetic kidney disease (DKD) remains the leading cause of chronic kidney disease (CKD) and dialysis in the developed world, and a major cause of premature mortality. Sodium-glucose cotransporter 2 (SGLT2) inhibitors confer cardiovascular and renal protection in many, but not all people with type 2 diabetes (T2D) (1). Yet, the mechanisms of these salutary cardiorenal benefits remain unclear, and are incompletely explained by the modest improvements in blood pressure, glycemic control, body weight, and serum uric acid (2). A better understanding of the mechanism of cardio-renal protection in response to SGLT2 inhibition is therefore needed to determine which patients would see the greatest benefit from this drug class and also how to augment the beneficial effects with complimentary drugs and/or lifestyle changes such as changes in dietary sodium intake.

As stated, the mechanisms by which SGLT2 inhibitors such as ertugliflozin induce cardiorenal benefits are uncertain. With respect to cardiovascular outcomes, hematocrit was statistically the most important mediator of heart failure reduction in the EMPA REG OUTCOME study (3). This increment in hematocrit likely reflects a reduction in plasma volume, which has originally been proposed to be caused by natriuresis. However, several trials (4,5), including the recent DAPASALT study (6) have indicated that SGLT-2 induced natriuresis is mild in terms of absolute amount of sodium excretion, is very transient (e.g. likely only present within 12 hour of the first dose) and therefore has been doubted to a) be the cause for the increment in hematocrit and b) to mediate the cardiovascular benefits.

This may be different for kidney outcomes, as SGLT2-induced changes in the location of tubular sodium reabsorption (thus even with neutral effects on total body sodium excretion), can largely impact kidney physiology. As such, more distal tubular sodium reabsorption (distal of the macula densa) triggers activation of tubuloglomerular feedback reducing glomerular pressure (7,8). In addition to changes in glomerular pressure, SGLT-2 induced changes in kidney physiology may drive changes in kidney oxygen metabolism.

From a metabolic point of view, the kidneys are highly active and are second only to the heart with respect to oxygen (O_2) consumption per tissue mass. To sustain this activity, the kidneys rely on various substrates to generate adenosine triphosphate (ATP) including citrate, glutamine, glucose and free fatty acids (FFA) (9). The vast majority of ATP consumption in the kidney relates to sodium reabsorption, and glucosuria and single-nephron hyperfiltration results in increased filtered sodium and activity of Na^+/K^+ ATPase pump due to high tubular glucose and sodium reabsorption (10-12). In fact, animal models suggest that renal O_2 consumption is increased by 40% in all cortical segments and by 160% each in the S3 segment and medullary collecting duct (13-17). In animal models, SGLT2 inhibition attenuates single-nephron hyperfiltration (18) and have also been shown to improve renal oxygenation and ameliorate renal hypoxia (19). Thus the SGLT2-induced renal benefit may be driven by improvements in renal oxygen availability. In addition, changes in tubular sodium reabsorption increase renal EPO production, which, through enhanced erythropoiesis, is the most likely explanation for the increase in hematocrit.

Thus, whether SGLT2 inhibitors modulate natriuresis or not, changes in tubular sodium handling lead to reduction of hyperfiltration, renal oxygen consumption and enhanced EPO secretion that collectively are thought to drive beneficial cardiorenal outcomes, amongst several hypotheses; effects that may be dependent of the amount of dietary sodium intake as argued below.

SGLT2 inhibitors may alter other aspect of sodium homeostasis as well. People with T2D are known to be sensitive to excess sodium intake, which may results in extracellular volume (ECV) expansion and hypertension. This is different for healthy, lean people in whom

increased salt intake usually does not increase blood pressure. These individuals are termed sodium-resistant (20,21). High-sodium intake suppresses SGLT2 expression leading to natriuresis. An effect not observed in rodents with hyperglycemia (22). Thus, SGLT2 inhibition during high-sodium diet could restore this response.
While it was previously thought that sodium sensitivity is exclusively driven by differences in renal sodium excretion, in salt-resistant people, sodium may bind non-osmotically in tissues such as endothelial surface layers and the glycocalyx, preventing water retention and hypertension (23). Integrity of the endothelial surface layer is important for vascular dynamics, and glycocalyx injuries have been implicated in the progression of DKD and cardiovascular disease. Interestingly, the SGLT2 inhibitor empagliflozin was shown to decrease tissue sodium content as measured by MRI, which could theoretically reduce non-osmotic sodium storage (24). On the other hand, SGTL2-inhibitor induced improvements in endothelial function could drive opposite effects with improved non-osmotic sodium storage. Currently, the effects of SGLT2 inhibition on sodium sensitivity are unknown, while its effect on sodium sensitivity may become very relevant in people that consume excess sodium. Given the blood pressure lowering effects of SGLT2-inhibitors, that may be driven by improvements in endothelial function, one could hypothesize that SGLT2 reduce sodium sensitivity.

Dietary sodium intake may modulate the effects of SGLT2 on the kidney and beyond through several mechanisms, as excessive sodium intake has kidney hemodynamic effects. As such, high sodium intake has been shown to increase GFR and effective renal plasma flow (ERPF) in normoglycemic individuals, while blood pressure (MAP) increased to a smaller extent. Given that renal vascular resistance = MAP/ERPF, this parameter declined. Filtration fraction (FF=GFR/ERPF) increased in the overweight individuals, but not in lean persons (25). As people with T2D are almost all overweight or obese and are usually sensitive to the effects of high-sodium intake (26), the renal hemodynamic effects of SGLT2 inhibitors could be off-set by high sodium intake. This could be secondary to impairment of tubuloglomerular feedback as shown recently for ertugliflozin in rodent studies (27). In addition, given the potential beneficial effect of SGLT2 inhibitors on kidney oxygenation, large amounts of filtered sodium could off-set this mechanisms of action.

Observations have been done with this reasoning for RAAS blockers. They are renoprotective in people with diabetes, likely secondary to reducing glomerular pressure through efferent vasodilation and lowering of filtration fraction (26). In people with diabetic kidney disease, this renoprotective effect was markedly affected by sodium consumption, with better protection afforded with low-sodium intake (27,28). Also for vitamin D (29) and neprilysin (30), interactions with sodium diet have been observed, with less efficacy at higher sodium intake. Based on these observations, it is plausible that baseline sodium intake is an important mediator for the kidney hemodynamic response and kidney protective effect of ertugliflozin.

In conclusion, although SGLT2 inhibitors mitigate cardiorenal risk in people with T2D, the protective effects are incomplete in the majority of people, which may be drive due to co-medication and dietary factors such as sodium intake. Therefore, carefully designed mechanistic trials are needed to better understand the interplay between ertugliflozin and salt intake. Studies focusing on these interactions will allow researchers to assess the effects of SGLT2 inhibitors in combination with other drugs that affect sodium homeostasis and potentially modify salt intake to maximize treatment response. In addition, such studies may also help to explain differences observed between (outcome) trials, which have included different ethnicities that may also contribute to differences observed in renal outcomes due to differences in dietary habits.

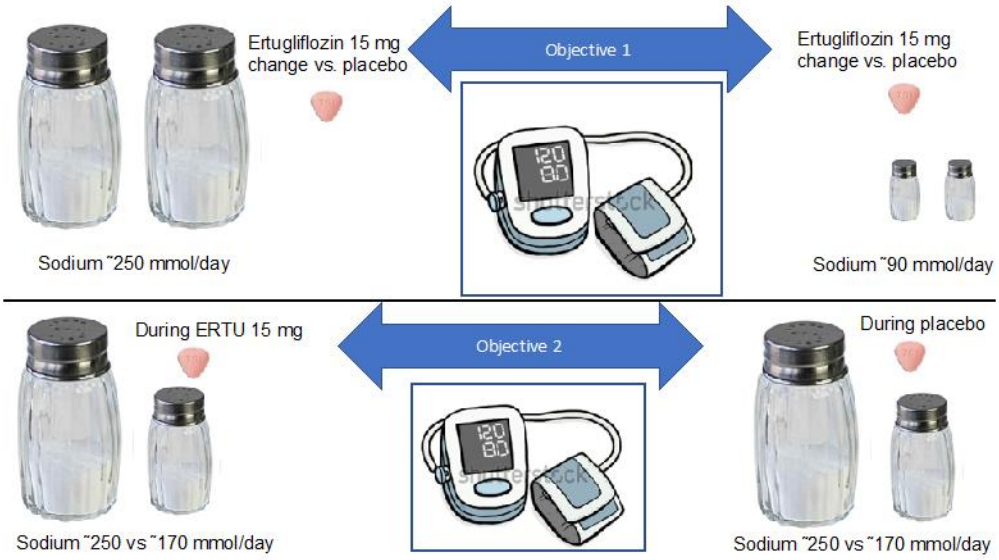
2. OBJECTIVES

2.1 Primary objective:

1: To investigate the modifying effects of WHO-recommended sodium intake (90 mmol per day) vs. high sodium intake (targeted at 250 mmol per day) on the effect of ertugliflozin 15 mg daily, versus placebo, on 24-hour blood pressure in overweight/obese adults with type 2 diabetes.

2.2 Secondary objective:

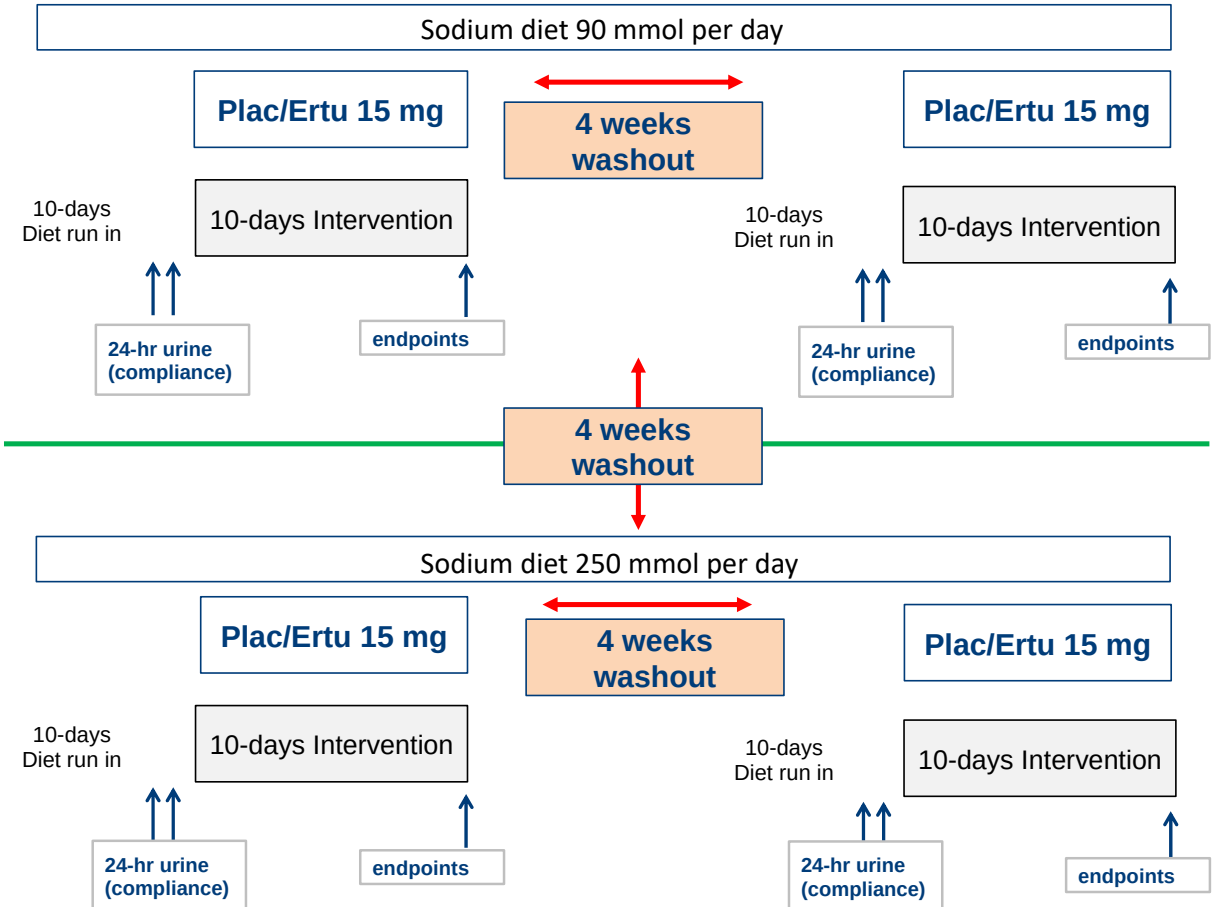
To investigate the efficacy of ertugliflozin 15 mg daily, versus placebo, in overweight/obese adults with type 2 diabetes to reduce the hypertensive effects of a high-sodium diet (250 mmol per day) versus 24-hour blood pressure measurement during participant's normal diet (170 mmol/per day) obtained at screening visit.



3. STUDY DESIGN

The study is a bi-center randomized, placebo-controlled, crossover intervention study. Total number of included participants is 34, which undergo 4 conditions in randomized order (randomization list generated by computer and supervised by pharmacy; blinded to investigator). While the treatment will be blinded for all participants, the sodium interventions are open-label. Study design is shown in the figure below.

The 4 conditions are:
1) low-sodium diet; placebo
2) low-sodium diet; ertugliflozin 15 once daily
3) high-sodium diet; placebo
4) high-sodium diet; ertugliflozin 15 mg once daily



4. STUDY POPULATION

4.1 Population (base)

Participants will be recruited from the database at Amsterdam Diabetes Center at Amsterdam UMC and by advertisement in local papers. This database has currently over 500 individuals with diabetes that have agreed to be approached for new studies and that have been phenotyped in dept. University of Colorado has a similar database ensuring rapid inclusion of required participants.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Adults with previously diagnosed T2DM according to American Diabetes Association (ADA) criteria
- HbA1c 6.5-10%
- Age 18 – 85 years of age
- Overweight or obese with BMI: >25 kg/m2
- We will make every effort to enrol participants of all races/ethnicities."
- Both sexes (females must be post-menopausal; no menses >1 year; in case of doubt, Follicle-Stimulating Hormone (FSH) will be determined with cut-off defined as >31 U/L)
- Ability to provide signed and dated, written informed consent prior to any study procedures
- Estimated GFR 60-90 ml/min/1.73m2 by CKD-EPI matching the eGFR range of most participants in VERTIS-CV
- Sodium intake at baseline < 200 mmol/day
- UACR < 30 mg/mmol
- All participants need to be on a stable dose of diabetes medication, including Metformin, SU, DPP4-inhibitors, or insulin.
- Participants suffering from hypertension need to be on a stable dose of RAS inhibitors. In case RAS inhibition is not tolerated, the participant should to be on a stable dose of other antihypertensive treatment.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- History of unstable or rapidly progressing renal disease

- Estimated GFR <60 mL/min/1.73m2 or eGFR > 90 mL/min/1.73m2 determined by CKD-EPI
- UACR > 30 mg/mmol
- Current/chronic use of the following medication: SGLT2 inhibitors, TZD, GLP-1RA, glucocorticoids, immune suppressants, antimicrobial agents, chemotherapeutics Participants should be on a stable dose of antipsychotics, tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs). Subjects on diuretics will only be excluded when these drugs cannot be stopped for the duration of the study.
- Chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) will not be allowed, unless used as incidental medication (1-2 tablets) for non-chronic indications (i.e. sports injury, headache or back ache). However, no such drug can be taken within a timeframe of 2 weeks prior to renal testing
- History of diabetic ketoacidosis (DKA) requiring medical intervention (e.g. emergency room visit and/or hospitalization) within 1 month prior to the Screening visit.
- Current urinary tract infection and active nephritis
- Recent (<6 months) history of cardiovascular disease, including:
  - Acute coronary syndrome
  - Chronic heart failure (New York Heart Association grade II-IV)
  - Stroke or transient ischemic neurologic disorder
- Severe hepatic insufficiency and/or significant abnormal liver function defined as aspartate aminotransferase (AST) >3x upper limit of normal (ULN) and/or alanine aminotransferase (ALT) >3x ULN
- Active malignancy. History of malignancy is allowed unless the participant still has active treatment other than hormonal therapy.• History of or actual severe mental disease
- Substance abuse (alcohol: defined as >4 units/day)
- Allergy to any of the agents used in the study
- Individuals who are investigator site personnel, directly affiliated with the study, or are immediate (spouse, parent, child, or sibling, whether biological or legally adopted) family of investigator site personnel directly affiliated with the study
- Inability to understand the study protocol or give informed consent

4.4 Sample size calculation

Based on previous studies in patients treated with SGLT2 inhibitors, we expect a change in systolic blood pressure of 6-8 mmHg during normal sodium intake (150 mmol/day) (6,7). Based on our hypothesis, this effect is increased on a low-sodium intake and reduced during high-sodium intake.

We expect a difference of 4 mmHg between the placebo-controlled effects of ertugliflozin on a low- versus high-sodium diet, with a conservative SD on this difference of 8 mmHg.

A total of 34 patients completing the study provide 80% power at a two-sided alpha of 0.05, to detect a 4 mmHg difference with the indicated SD of 8 mmHg. Drop-outs will be replaced.

Based on the RED study (7) and DAPASALT(6) study, the power needed to detect changes in GFR and hematocrit are of sufficient magnitude and therefore we expect sufficient power for these parameters as well. **We will include 34 individuals.**

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

In addition to their ongoing oral glucose-lowering medication (metformin or a SU-derivative) and drugs for cardiovascular risk management (e.g. ARB and/or statin), subjects will randomly be assigned to receive ertugliflozin 15mg or a matched placebo treatment during the first intervention phase of the study. Following a wash-out phase the participants will than start with the second intervention phase and receive the other study medication (see table 1).

Table 1. Overview treatment allocation

Product and dosage	Dosage form and strength	Manufacturer
Ertugliflozin 15mg QD	15 mg tablets (orally)	Merck
Placebo ertugliflozin-matching tablet QD	Matched tablets (orally)	Merck

The study medication (ertugliflozin and matching placebo) will be provided by Merck. The pharmacy department of Amsterdam UMC, location VUMC, will be responsible for randomization and distribution of study medication.

Study medication details: ertugliflozin (Steglatro® by Merck Sharp& Dohme BV., USA). Ertugliflozin L-pyroglutamic acid: (1S,2S,3S,4R,5S)-5-(4-chloro- 3-(4-ethoxybenzyl)phenyl)-1- (hydroxymethyl)-6,8- dioxabicyclo[3.2.1]octane-2,3,4-triol, compound with (2S)-5- oxopyrrolidine-2- carboxylic acid.

5.2 Use of co-intervention (if applicable)

During the treatment phases, participants will be subjected to a sodium diet. While a regular sodium intake in the Netherlands, based on previous studies and literature, is around 170 mmol/day; we will coach participants by providing food lists and counselling, to lower their intake to WHO-recommended amounts of 90 mmol/day. This will be done during 10 days prior to start study medication (compliance checked by 24-hour sodium analysis) and during the 10-day intervention. Thus for a total of 20 days per treatment block. Low-sodium intake will be effected during both ertugliflozin and placebo. Similarly, using dietary counselling, participants will be instructed to follow a high-sodium diet aimed at 250 mmol/day.

5.3 Escape medication

Not applicable

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Study medication details: ertugliflozin (Steglatro® by Merck Sharp& Dohme BV., USA). Ertugliflozin L-pyroglutamic acid: (1S,2S,3S,4R,5S)-5-(4-chloro- 3-(4-ethoxybenzyl)phenyl)-1- (hydroxymethyl)-6,8- dioxabicyclo[3.2.1]octane-2,3,4-triol, compound with (2S)-5- oxopyrrolidine-2- carboxylic acid.

6.2 Summary of findings from non-clinical studies

Please refer to the IB

6.3 Summary of findings from clinical studies

Please refer to the IB

6.4 Summary of known and potential risks and benefits

Please refer to the IB.

In addition, a structured risk analysis is provided in chapter 13.

6.5 Description and justification of route of administration and dosage

The standard clinical dosage is 15 mg once daily. It is oral administration. Please refer to IB.

6.6 Dosages, dosage modifications and method of administration

The dosage in this study is 15 mg once daily, oral. This is conform the manufacturer's instructions and what has been extensively studies in clinical trials.

6.7 Preparation and labelling of Investigational Medicinal Product

The investigational product (ertugliflozin and placebo) are provided by MSD according to GMP production. Labelling will be done in collaboration with the trial pharmacy at Amsterdam UMC.

6.8 Drug accountability

Medication is shipped to both study sites by MSD and will be received by the trial pharmacy at Amsterdam UMC according to guidelines.

Randomization

The treatment assignment will be performed by the trial pharmacists of Amsterdam UMC-location VUmc (who have ample experience with this process), in order to maintain blinding for the investigator, site personnel, and patients. A randomization sequence will be generated and sent through an automated system (Castor EDC) by the investigator to the trial pharmacy, ensuring that the investigator remains blinded.

Labeling, Drug dispensation, and Accountability

Merck will provide the trial drug and the placebo. The trial products will be labelled with participant identifying information to our institutional trial pharmacist, who subsequently will store the trial products according to prevailing guidance until they are needed. The trial pharmacist will deliver the study medication to the research personnel (who remain blinded). These will then dispense the study medication to the participants at the designated visits, combined with extensive instructions on use and administration of the study drug. The number of daily dosages dispensed will be sufficient to cover the complete expenditure of the study. No trial products will be dispensed to any person not enrolled in the trial.

On the designated days, the participants will bring their trial medication. In order to perform proper drug accountability, the investigator will keep track of all received, used and unused trial products and -if possible- all empty packaging. Non-used medication will be returned to the pharmacy department where it will be destroyed.

Drug storage

Ertugliflozin does not require any special storage conditions (Agency, 2018). Nevertheless, when not distributed to participants, drugs will be stored at the institutional trial pharmacy. Proper storage conditions will be ensured and records and evaluations will be kept as required by International Conference on Harmonisation – Good Clinical Practice (ICH-GCP).

Blinding and Unblinding

Throughout the study duration, all study personnel will remain blinded with regard to the medication used. The randomization –in order to achieve blinding for study personnel- will be performed by the trial pharmacist of the Amsterdam UMC-VUmc. The investigator will receive the subjects blinding information concerning the study medication in the form of a sealed envelope; this envelope will be stored on a secure location in the Diabetes Center Amsterdam UMC-VUmc. The blind shall not be broken by the investigator unless information concerning the study medication is necessary for the medical treatment of the subjects. Procedures regarding emergency unblinding are described in Chapter 9. If the investigator is unblinded, study medication will be stopped immediately in addition to the withdrawal of the study subject from the study. At the end of the study, following the last patients last visit and database lock (which will only occur after medical / scientific review), the blind will be broken by the trial pharmacist.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Iohexol (Omnipaque® by GE Healthcare B.V.) (1)

Supplied as: 200 mL ampoules with a clear, colorless solution containing 300 mg iohexol per mL.

Approved agent: Approved by the Dutch drug authority (College ter Beoordeling van Geneesmiddelen; RVG 09821)

Action: Iohexol is a non-ionic, monomeric, triiodinated, water-soluble substance mostly used as X-ray contrast medium. In the concentration of 140 mg I/ml it is isotonic with blood and tissue fluid. Close to 100 per cent of the intravenously injected iohexol is excreted unchanged through the kidneys within 24 hours in patients with normal renal function. The maximum urinary concentration of iohexol appears within approximately 1 hour after injection. No metabolites have been detected. The protein binding of iohexol is very low (less than 2%).

Side effects: The risk of serious reactions in connection with use of Omnipaque is regarded as minor. However, iodinated contrast media may provoke serious, life-threatening, fatal anaphylactic/anaphylactoid reactions or other manifestations of hypersensitivity.

Dosing: For the steady-state iohexol-clearance test, an initial bolus dose is given, followed by continuous infusion with a dose calculated to maintain the plasma concentration between 100 mg/L and 150 mg/L.

Storage: Iohexol can be stored up to 3 years below 30°C.

Sodium-4 amino hippurate (PAH, Basic Pharma Manufacturing BV) (2)

Supplied as: PAH is supplied as single use vials of 10mL vials containing sterile 20% (2g/10mL) of 4-Aminohippuric acid dissolved with one equivalent of sodium hydroxide using water for injection.

Approved agent: An authorized PAH, USP Grade, manufactured by Merck, was available for research for the estaimation of ERPF. However, the authorized product was removed from the market in the late ‘90s/ early 20’s and is no longer available for use in clinical trials. PAH manufactured by Basic Pharma does not yet have marketing authorization in an ICH country.

Action: PAH is a derivative of hippuric acid, which is derived from the amino acid glycine. It is inert in normal doses.

Side effects: Extremely large doses may cause osmotic diuresis. Some patients may have a sensation of warmth, or the desire to urinate or defaecate during or shortly following the infusion. Allergic and anaphylactic reactions have been reported with the PAH produced via Merck but were very uncommon.

Dosing: During one PAH-clearance test, an initial bolus will be given followed by a continuous infusion with a dose calculated to maintain the plasma concentration between 15mg/L and 20mg/L.

Storage: PAH will be stored at room temperature protected from light.

7.2 Summary of findings from non-clinical studies

Please refer to IB

7.3 Summary of findings from clinical studies

Please refer to IB

7.4 Summary of known and potential risks and benefits

See above and in the structured risk analysis. At our research centre at Amsterdam UMC we have done > 500 iohexol and PAH clearances in the last 5 years and not encountered a single problem.

7.5 Description and justification of route of administration and dosage

Intravenous usage. Dosage are indicated above.

7.6 Dosages, dosage modifications and method of administration

Please see above.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable

7.8 Drug accountability

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

To investigate the modifying effects of WHO-recommended sodium intake (90 mmol per day) vs. high sodium intake (targeted at 250 mmol per day) on the effect of ertugliflozin 15 mg daily, versus placebo, on 24-hour blood pressure in overweight/obese adults with type 2 diabetes.

8.1.2 Secondary study parameters/endpoints (if applicable)

To investigate the efficacy of ertugliflozin 15 mg daily, versus placebo, in overweight/obese adults with type 2 diabetes to reduce the hypertensive effects of a high-sodium diet (250 mmol per day) versus 24-hour blood pressure measurement during participant's normal diet (170 mmol/per day) obtained at screening.

8.1.3 Other study parameters (if applicable)

To investigate the modifying effects of WHO-recommended sodium intake (90 mmol per day) vs. high sodium intake (targeted at 250 mmol per day) on the effect of ertugliflozin 15 mg daily, versus placebo, on the following parameters in overweight/obese adults with type 2 diabetes:

- GFR by iohexol clearance
- Hematocrit
- Office blood pressure
- Kidney oxygenation
- Parameters of intra kidney hemodynamic function including ERPF by p-aminohippurate (PAH) clearance and renal vascular resistance
- Body anthropometrics
- Fasting plasma glucose
- Albumin excretion rate (24 hour)
- 24-hr urinary glucose excretion
- Urinary and plasma biomarkers

8.2 Randomisation, blinding and treatment allocation

The study is blinded with ertugliflozin and matching placebo's being provided by MSD. The investigator will send a computer-generated randomization list (block randomization of 4) via an automated system (Castor EDC) to the trial pharmacy of Amsterdam UMC. In this way, all study participants and trial personnel remain blinded at all times. Unblinding will only occur in a medical emergency. The sodium diets are

of course open label. Each participant receives sufficient tablets (ertugliflozin or matching placebo) for each treatment period of n=10 days.

8.3 Study procedures

Recruitment

Potential participants will be recruited by researcher physicians involved in this trial using methods that are established practice for all human studies in the Diabetes Center Amsterdam UMC-VUMC:

1) Via advertisements in (local) newspapers
2) Use of a Diabetes Center database to approach volunteers from previous studies, who have given written informed consent to be available for future studies
3) Through the Diabetes Center website (www.diabetescentrum.nl)
4) Via general practitioner offices with whom we have collaborations., Potential participants will be approached by the general practitioner
5) Where possible, subjects will be recruited from the out-patient clinic of the Diabetes Center / Department of Internal Medicine Amsterdam UMC-VUmc or affiliated hospitals / pharmacies
6) Online advertising directed at patient support groups

In case of a positive response, the information letter and informed consent forms will be sent to these individuals. They will then be contacted by telephone by the research physician to answer any remaining questions and make an appointment for a screening visit if the individual wants to participate.

Screening and eligibility (V1 and T1)

After giving extensive oral and written information, and sufficient time to consider study participation (minimally one week), a written informed consent form will be obtained from the subjects before screening. Once the participant signs the informed consent form he/she will be assigned a unique study number, which will be used for identification.

The screening procedure will consist of obtaining an extensive medical history (including disease history, current medical conditions and use of medication, substance use, employment, education, family history), complete physical examination, drawing blood for hematologic and biochemistry testing (full blood count, glucose, HbA1c, creatinine, ALT, AST, gamma-glutamyl transferase (GGT), creatinine, albumin, and thyroid-stimulating hormone (TSH)), urine screening, urine creatinine and albumin, and a 12-lead electrocardiogram (ECG). If subjects are eligible, the investigator will inform them within two weeks after screening. Then, 24-hour urine and 24-hour blood pressure are obtained in order to be able to study hypothesis 2 (effect of ertugliflozin on sodium sensitivity).

After confirmation of eligibility during screening, a 24-hr blood pressure measurement as well 24-hr sodium excretion is measured prior to the dietary blocks as detailed below. This 24-hr sodium and blood pressure measurement will be crucial for hypothesis 2. Urine containers and 24-hr blood pressure meters are provided to the patient and collected after recording.

Screening	Day 0	Day 1 (offered at home of participants to reduce travel
Medical history	X	
Physical examination	X	
Review of medication	X	
Office blood pressure	X	
Routine hematology and chemistry	X	
HbA1c	X	
Urinary dipstick	X	
Start 24-hr urine sodium collection	X	
Start 24-hr BP measurement	X	
Return 24-hr urine sodium collection		X
Return 24-hr BP measurement		X

Subsequently, 4 of the blocks of 20 days below are conducted in random order with differences in study medication (placebo versus ertugliflozin) and sodium diet (90 mmol/day versus 250 mmol/day). The washout is 4 weeks between the blocks. Please refer to the figure on page 11. Urine containers are provided to the patients.

A sodium diet is commenced (either aimed at 90 mmol/day or 250 mmol/day) and it is expected that after 10 days this is reached (+/- 20%, due to normal variation in sodium excretion). This will be assessed with two 24 hour urine collections. Subsequently, the diet is continued and treatment with the study medication commences. In the morning of day 9, 24-hour blood pressure recording starts. At the study visit at day 10 of treatment, endpoints are collected following an overnight fast of 10-hours at our clinical trial unit as indicated below. A flow chart of study activities is attached below.

Subject will arrive at Amsterdam UMC location AMC for multimetric MRI following an overnight fast. Subjects are then brought to Clinical Research Unit (CRU) location VUMC where the 24-hour urine collection is processed (in order to assess sodium

excretion and other variables of interest obtained from 24-hr urine collection). The 24-hour blood pressure recording is stopped. Two intravenous catheters will be placed in both forearms for blood sampling and for the administration of test-agents (e.g. iohexol and PAH). A total of 125 mL blood will be drawn during the day for this purpose. The renal protocol commences with measurement of GFR and effective renal plasma flow (ERPF) based on continuous infusion for 2.5.
During the infusion, several measurements will be performed including anthropometrics, bio-impedance analysis, blood pressure measurement and collection of samples for biomarker analysis.

	Day -10	Day -2	Day -1	Day 0	Day 3	Day 10
Start sodium-diet (continue until day 10)	X					
24-hr urine sodium (compliance)		X	X			
Start study medication (continue until day 10)				X		
Telephone call compliance					X	
Recent history						X
24-hr blood pressure						X
Measured GFR						X
ERPF						X
Intrarenal hemodynamic function						X
Office blood pressure						X
Hematocrit						X
Renal oxygenation (BOLD MRI)						X
RAAS components						X
Collection of biomarkers						X
Body anthropometrics						X
24-hr urine analysis (sodium, UACR)						X

Efficacy measurement more detailed:

1) **Height**. A height measuring board will be used to assess height. Patients will be asked to remove their footwear and headgear and to stand on the board with their feet together, heels against the back board and knees straight. The patient should be looking straight ahead, without tilting the head up, with eyes and ears at the same level. The measure arm will gently be moved down onto the head of the participant and height will be read in centimeters at the exact point.
2) **Weight**. Body weight will be recorded by a digital calibrated weighing scale on a firm, flat surface. Patient will be asked to remove their footwear, extra layers of clothing, jeweler and any items in his/her pockets before standing still on the weighing scale with arms placed on the side. Measurement will be recorded on the Case Report Form (CRF) to the nearest kilogram. No deduction for clothing will be permitted.
3) **Body Mass Index** (BMI). BMI will be derived from height and bodyweight by using the mathematical formula: BMI=[kg/[m]2.
4) **Waist circumference**. A constant tension tape will assess waist circumference with the patient in a standing position with the arms relaxed at the sides and without clothing, that is, directly over the skin. Measurement will be performed at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration.
5) **Body-impedance analysis**. To measure body fat content we will use the tetrapolar Soft Tissue Analyzer® (STA, Akern, Florence, Italy), which can perform Bio-Impedance Analysis (BIA). This technique measures the resistance in the body tissues when a 50 kHz AC electrical current is given between four electrodes. Resistance can be measured by placing two electrodes on the dorsal hand and two on the foot at the unilateral side of the body (Lukaski & Bolonchuk, 1988). Extracellular water (ECW) as a percentage of total body water (TBW) and body cell mass (BCM) will be calculated independent of weight and height.
6) **ECG**: A 12-leads ECG will be made in the fasting resting state during screening in order to assess cardiac conduction parameters. Heart rate on the ECG will be defined as the mean rate of 10 R-R intervals. PQ-time, QRS-time and QT-time will be calculated, QT-time will be corrected for heart rate by using the Bazett formula (C. Bazett, 2006). The cardiac vector and ECG morphology will be assessed.
7) **Blood pressure and heart rate** (office): Blood pressure and heart rate will be measured using an automatic oscillometric device (Dinamap®, GE Healthcare) After an acclimatization period of >5 minutes, blood pressure will be measured three times at the non-dominant arm, with the subject in a semi-recumbent position, using an appropriate cuff-size. The mean of the last 2 measurements will be used as final value (Mengden et al., 2010; Pickering et al., 2005).
8) **GFR** is measured by steady-state plasma iohexol concentrations measured at T=120 and T=150 as done in our previous approved protocols.

9) **ERPF** is measured by steady-state plasma PAH concentrations measured at T=120 and T=150 as done in our previous approved protocols.
10) **Filtration fraction** (GFR/ERPF) and renal vascular resistance (ERPF/mean arterial pressure) are calculated.
12) **24-hour blood pressure** is derived from Mobil-O-graph devices (24 ambulatory blood pressure monitors, The Netherlands), which will be available at both sites. Daytime systolic blood pressure (SBP), daytime diastolic blood pressure (SBP), nighttime SBP, daytime DBP and nocturnal dipping pattern (in mmHg) are recorded.
12) sodium sensitivity is calculated as following: changes in blood pressure due to the high-sodium diet versus diet at screening (estimated based on experience/previous trials at ˜165-170 mmol/day) and the effect on this parameter by ertugliflozin versus placebo.
13) **Multiparametric MRI** will be carried out to measure additional kidney physiology parameters. The multiparametric MRI is performed on a tesla 3 machine. The MR examination will last 45 minutes maximum. During the scans, heart rate and blood pressure will be monitored. Patients get hearing protection in the form of ear-plugs and headphones. During the examination, they are in contact with the technicians via the intercom. Throughout the examination, patients will receive breathing instructions, for example to hold their breath for 20 seconds maximum, in order to produce accurate images. The MRI exam includes BOLD MRI to measure oxygenation, but also ASL and phase contrast MRI to obtain renal artery blood flow.
- BOLD-MRI: BOLD-MRI will be performed in order to measure renal tissue oxygenation. BOLD MRI exploits the difference in magnetic properties between oxygenated and deoxygenated hemoglobin (Pruijm et al., 2018). Presence of deoxygenated hemoglobin will result in an accelerated signal decay on the BOLD images. Measurement of this signal decay results in a measure of the amount of deoxygenated hemoglobin, the apparent relaxation rate R2*, which inversely relates to level of tissue oxygenation.
-ASL: In ASL, the blood travelling through the aorta is magnetically labeled before acquiring an image (the “label” image) (Odudu et al., 2018). Other images are acquired without any label (the “control” image). The difference between the label and control image will only be around 2%, so this procedure is repeated several times. The average difference between those images can be used to obtain a perfusion map of the kidney. Phase-contrast MRI is done to obtain renal artery blood flow.
14) **serum biomarkers**: hematocrit, serum albumin, plasma glucose and additional serum biomarkers are measured in the fasted state
15) **24-hour urine albumin and glucose excretion** is measured. Additional material for urinary biomarkers of interest such as markers of inflammation and electrolytes is stored.
16) **Safety and tolerability**: recent history and focused physical examination is carried out to assess potential adverse effects of study medication.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. When participants decide to withdraw their participation, they will be asked to participate in 'early-term assessments'.

After subjects have entered the study, participation will be stopped in case of occurrence of any of the following:

- Participants own decision
- The occurrence of an AE or clinically significant laboratory change or abnormality that, in the judgment of the investigator, warrants withdrawal of the study
- Subjects need medical procedures that are not allowed in the protocol
- Subjects cannot undergo the procedures to investigate the research questions as outlined in this protocol
- Non-compliance
- In addition to these requirements for study drug discontinuation, the investigator should discontinue study participation for a given subject if, on balance, he thinks that continuation would be unbeneficial to the subjects well-being

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

Participants will be replaced to maintain sufficient power.

8.6 Follow-up of subjects withdrawn from treatment

If patients withdraw from the study, vital signs will be collected and recent history to confirm safe withdrawal from the study. There is no harm in suddenly stopping study medication, although glucose levels may increase mildly.

8.7 Premature termination of the study

I do not expect premature termination of the study. No interim analysis for efficacy is performed. Ertugliflozin is a drug with a well-established safety profile, and no new side effects are expected to be found in this study with 2 blocks of 10 days of active treatment. Obviously, if SAE are encountered that may be related to the study medication, a careful decision will be made to halt the study prematurely.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (Aes)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Because this study is considered to be low-risk, as agreed by the Ethical Committee, SAE's will be recorded and reported yearly through the web portal ToetsingOnline to the accredited METC that approved the protocol.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

The site at Colorado University (PI Dr Bjornstad) will report all SAEs and SUSARs to the local competent authority according to the requirements of that Member State. In addition, they will inform the sponsor/coordinating investigator of the study (Dr. van Raalte) and MSD within a single day.

Please describe also the method of breaking the code for SUSAR reporting:

The pharmacy of each participating center performs the randomisation of subjects to the treatment categories. In the event of an emergency, the local pharmacy provides the local researcher with information concerning the treatment of the subject of the SUSAR. Unblinding is not to be performed for any reason other than an emergency where unblinding is required and should be reported in the CRF. The local investigator must also immediately inform the project leader and the principal investigator about the unblinding procedure. If it does not compromise the welfare of the participant, a close-out visit which includes drawing of blood samples for safety or pharmacodynamic assessments will be performed.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All Aes will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

10. STATISTICAL ANALYSIS

Sample size calculations

The primary endpoint of the study is to investigate the modifying effects of WHO-recommended sodium intake (90 mmol per day) vs. high sodium intake (targeted at 250 mmol per day) on the effect of ertugliflozin 15 mg daily, versus placebo, on 24-hour blood pressure in overweight/obese adults with type 2 diabetes.

Based on previous studies in patients treated with SGLT2 inhibitors, we expect a change in systolic blood pressure of 6-8 mmHg during normal sodium intake (150 mmol/day) [PMID: 33318125; PMID: 23668478]. Based on our hypothesis, this effect is increased on a low-sodium intake and reduced during high-sodium intake.

We expect a difference of 4 mmHg between the placebo-controlled effects of ertugliflozin on a low- versus high-sodium diet, with a conservative SD on this difference of 8 mmHg.

A total of 34 patients completing the study provide 80% power at a two-sided alpha of 0.05, to detect a 4 mmHg difference with the indicated SD of 8 mmHg. Drop-outs will be replaced. Based on the RED study and DAPASALT study, the power needed to detect changes in eGFR and hematocrit are of sufficient magnitude and therefore we expect sufficient power for these parameters as well. The calculation is based on a paired design. The sample size was calculated using Stata-Software (v. 11.2, College station, TX, USA).

10.1 Primary study parameter(s)

General considerations

1. All tests of differences between treatments will be conducted at a two-sided significance level of <0.05. In general, the null hypothesis (H0: no difference exists between treatment with the study drug and placebo) intervention) will be tested against the hypothesis H1: a difference exists between treatment with the study drug and placebo), with respect to the underlying parameter of interest.
2. All variables will be expressed as mean ± SEM when normally distributed, or –in case of non-normal distribution- be expressed as median and interquartile range (IQR). Log transformation will be applied to obtain normal distribution as appropriate for the statistical tests.

3. In case of missing data, a multiple imputation technique will be applied and compared with complete case analysis.

Analysis population

The efficacy analysis population will be based on the per protocol (PP) principle – i.e. all subjects who completed the entire treatment period using the treatment as originally allocated. Safety analysis will be performed in all patients who received one or more doses of the study medication.

Statistical analysis of study endpoints

We will use a two-way ANOVA to evaluate the main effects of diet and treatment as well as an interaction between ertugliflozin vs. placebo during high and low-sodium intake on the main outcomes. Appropriate covariates may be added to the model. Because of the crossover design of our trial, we will assess the carryover effect by including the sequence allocation as a factor in the mixed model. If a carryover effect will be detected ($p<0.1$), only the first study period will be analysed (treating it as a parallel randomised controlled trial).

For possible confounders, we will test whether there is an association with both the dependent and independent variable ($p <0.1$). If this is the case, this confounder will be added to the model as covariate. Since all outcome variables – primary, secondary and exploratory – are continuous, this statistical technique can be applied.

The large amount of secondary/exploratory endpoints potentially justifies the use of corrections for multiple tests. However, there is an increasing debate on whether this should be done, because of the increased risk of type 2 errors which could lead to prematurely discarding of potential useful observations (Rothman, 1990). As recently pointed out (Streiner & Norman, 2011), in a hypothesis-generating study – such as the current study – correction for multiple outcomes can be detrimental.

10.2 Secondary study parameter(s)

The secondary endpoint is analysed by comparing the effects of high-sodium on blood pressure increments during placebo and ertugliflozin treatment. An ANOVA analysis will be carried out to test significance between different conditions.

10.3 Other study parameters

All exploratory variables are analysed as described under 10.1. For exploratory parameters, no correction for multiple testing will be conducted and the exploratory nature of the data will be stressed when the data are reported in a manuscript as our group has done previously.

10.4 Interim analysis (if applicable)

No interim analysis will be performed

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, 2013), in accordance with the Medical Research Involving Human Subjects Act (WMO), and in accordance to the European Clinical Trial Regulation (ECTR). The investigator will ensure that all aspects of the institutional ERB review are conducted in accordance with current institutional, local and national regulations.

11.2 Recruitment and consent

Patients will be enrolled to the trial from centers in the Netherlands (1 center) and the United States (1 center). Potential participants will be approached through (a) the diabetescenter database, which has registered participants that have given consent to be approached for new studies by their informed consent form of a former stud, (b) adds online or in local newspapers, or (c) through their own physician.

(a) Former participcants of former studies, that gave their consent to be approached for new studies, can be contacted by the research physician. In case of a positive respons, the participant information letter wil be send out. After a minimum of a week, in which the possible participant can read the information and formulate questions, the research physician will contact the possible participant again and answer any questions. If the possible participant wishes to participate, an appointment will be made for the screening visit. During this visit there again is room for explanation of the study and questions. Than, before the commencement of any study procedures, the informed consent form will be signed in twofold by the participant and the research physician.
(b) People who have read the add and have interest in participation of the study can contact the research physician by mail or by phone. After the inital contact has been made, the procedure is similar tot he procedure described above. The research physician will explain the details of the study and the PIF will be send. After at least a week, there will be a second contact moment and room for questions. In case of a positive response, the possible participant will be invited to the hospital for a screening visit. During this visit there again is room for explanation of the study and questions. Than, before the commencement of any study procedures, the informed consent form will be signed in twofold by the participant and the research physician.
(c) A doctor that is familiar with current study, can ask his/her own patients if they have interest in participation in the study and if the research physician can contact them. In casse of a positive respons, the procedure is similar tot he procedure described above. The research physician will contact the possible participant and explain the details of the study and the PIF will be send. After at least a week, there will be a second contact moment and room for questions. If the possible participant wishes to participate he/she will be invited to the hospital for a screening visit. During this visit there again is room for explanation of the study and questions. Than, before the commencement of any study procedures, the informed consent form will be signed in twofold by the

participant and the research physician.

11.3 Benefits and risks assessment, group relatedness

All participants will receive ertugliflozin, an SGLT-2 inhibitor which is approved for blood-glucose lowering treatment in T2DM patients. Based on currently available data from thousands of patient years, the drug has shown to be safe. In addition to glucose-lowering, SGLT-2 inhibitors have other beneficial effects, by inducing a decrease in blood pressure and body weight for instance. Clinically, SGLT2 inhibitors prevent kidney function decline, hospitalization for heart failure and cardiovascular events after longer-term use.
SGLT2 inhibitors have side effects. The most common side effect are genital mycotic infections, which can be treated with a topical antimycotic crème. Other side effects include polyuria, frequent voiding and nycturia.

As in all drug intervention trials, in this study, we will closely monitor patients for adverse drug and study events. Participants can contact the research staff 24 hours a day. It should be noted that most of these side effects rarely occur in such a short treatment duration as in the current study. There is no risk of a short-term change in sodium diet on long-term health outcomes. In addition, effects of high-sodium diet [e.g. on blood pressure] are closely monitored.

11.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.5 Incentives

Patients will receive restitution of all costs for transportation and a honorarium of 500 euro for participating. In case of premature termination, the reimbursement of the participant will be based on the actual duration of participation in proportion to the planned duration of participation.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

In accordance with the Dutch law 'Algemene verordening gegevensbescherming' (AVG; General Privacy Law) and GCP all patient data will be handled confidentially. Therefore, all participants at Vumc will get a study code ('DES-0xx'). The code list, with both identifier data and study number, will be stored securely on the server of the VUMC, protected by passwords only known by the responsible research physicians and principal investigator.

All subject data will be recorded on printed CRF by the local investigator and/or personnel that is instructed in performing these administrative tasks and study procedures. In the latter case, data will always be reviewed by the investigator. All subject related data will be stored for the legally required period of 25 years. With regard to the body material, data will be stored for a maximum of 5 years and will only be used for the research questions within the scope of current research. Subject data, both encoded (e.g. CRFs) or not (e.g. source documents), will always be stored securely, in a locked cabinet in the Diabetes Center VUMC or on password secured computers.

For the site in Colorado, the same principles are maintained as indicated here. Data from the US site will be sent to Amsterdam UMC, in an anonymized matter (using a study code DES-1xx). This has been described in the study contract between sites. Vice versa, data will not be send from the Dutch site to the US.

Monitoring and Quality Assurance

The study will be monitored by an appointed monitor of the VU ethical review board. The monitor will conduct (a frequency of) quality assessments in accordance to the guidelines of the institution. Monitoring at Colorado University is carried along the same principles with a local monitor.

12.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.3 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.4 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.5 Public disclosure and publication policy

Publication will be in accordance with the basic principels of Central Committee on Research involving Human Subjects (CCMO) statement on publication policy.

As described in the contract with MSD, the sponsor has full rights to publish the data unreservedly. This concerns both abstracts that are sent for presentation at conferences and manuscript for publication in high-impact peer-reviewed journals. The Amsterdam and Denver site will co-publish the data under the strong collaboration between PI van Raalte PI Bjornstad. Please refer to the contract with MSD for details.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Study medication

Participants will receive ertugliflozin 15 mg, an SGLT-2 inhibitor which is approved for blood-glucose lowering treatment in T2DM patients, and based on currently available data, is considered to be safe. Furthermore, SGLT-2 inhibitors may have other beneficial effects in general and are associated with a decrease in blood pressure and body weight. In large-sized cardiovascular outcome study, ertugliflozin was shown to have beneficial effects on cardiovascular outcomes and chronic kidney disease.

The most common adverse effects for ertugliflozin are genital mycotic infections and to a lesser extent pruritus, polyuria, frequent voiding and nycturia. However, genital mycotic infections usually occur after prolonged treatment, at least weeks. For canagliflozin, not ertugliflozin, increased amputations were reported in one study, but not in people with kidney disease, mostly in people that had previous amputations.

Hypoglycemia: SGLT2 inhibitors do not cause hypoglycemia as they do not increase insulin secretion. In people without diabetes, SGLT2 inhibitors do not cause hypoglycemia. When combined with drugs as insulin and sulfonylurea, SGLT2 inhibitors may cause hypoglycemia, particularly in people with normal eGFR.

Keto-acidosis: this has been reported in people with type 1 diabetes,[28] but is rare in people with type 2 diabetes with only a few cases reported worldwide.[28]

Sodium diet:

Participants will undergo two periods of increased sodium intake (of 20 days each). While higher sodium intake, may increase blood pressure, it is usually not associated with adverse effects. Of note, sodium intake in the Netherlands is really high, therefore, for some people it may be their normal intake. Lowering sodium intake is not related to any health risk.

a. Level of knowledge about mechanism of action

Regarding side effects, they have a well-defined mechanism of action:

1. Genitourinary mycotic infections: due to SGLT2-induced glucosuria, urine provides a better culture media for mycotic infections.
2. Hypoglycaemia: higher risk in people with concomitant insulin therapy and/or sulfonylurea. Due to glucosuria, the amount of circulation can be inappropriate leading to hypoglycaemia.
3. Keto-acidosis: due to lowering of insulin concentrations due to glucosuria, and due to SGLT2-induced increment in glucagon concentrations, ketogenesis is enhanced. The nature of this mechanism facilitates clear actions to mitigate this effect as detailed below.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Please refer to the IB

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Please refer to the IB

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Please refer to the IB

e. Analysis of potential effect

The dosage of 10 mg has good safety profile. Please refer to the IB.

f. Pharmacokinetic considerations

The dosage of 10 mg has good safety profile. Please refer to the IB.

g. Study population

The included participants are people with type 2 diabetes that are otherwise healthy. They are similar to the patients included in the ertugliflozin trials, making the effects on our population predictable.

h. Interaction with other products

Please refer to the IB

i. Predictability of effect

Please refer to the IB

j. Can effects be managed?

The most common side effect of SGLT2 inhibition therapy is genital mycotic infections. They occur usually after more chronic treatment. The incidence is increased 2.47-fold compared to placebo. However, a recent study in people with heart failure indicated that this may be reduced to 1.7% (incidence rate per 100 patient years 1.4) versus 0.7% in the placebo group

(incidence rate per 100 patient years 0.5) when proper instructions with respect to hygiene are provided, as will be done in our study. In addition, it is easily treated with a topical drug. The most feared side effect is euglycemic diabetic ketoacidosis (DKA). Due to lowering of endogenous or exogenous insulin levels, people with type 1 diabetes showed marked increases in euglycemic DKA despite controlled trial setting. For this reason, SGLT2 inhibitors are only in exceptional situations given to people with type 1 diabetes. In people with type 2 diabetes, euglycemic DKA is extremely rare, approximately 1 case per 1000 patient years. This likely concerns misclassified type 2 diabetes patients in whom insulin was stopped or reduced. To mitigate euglycemic DKA risk in our study, type 1 diabetes patients are excluded in our study, as well as individuals with diabetes and BMI <25 kg/m2 as they phenotypically resemble type 1 diabetes patients. To prevent hypoglycemia, insulin dosages are lowered by the study team prior to initiation of the study drug if indicated. The study team consists of lead experts in the field of diabetology with ample experience in dosing glucose-lowering drugs. For the sodium diet we will monitor blood pressure when complaints are expressed by the study participant.

13.2 Synthesis

Although there will be no medical benefit for participants of this study, we feel that the risk of the study protocol is moderate. The drug is well-tolerated in thousands and thousands of patient years with side effects being predictable, preventable and treatable. The effects of the sodium diet also do not pose a risk. In addition, the participants may receive education how to lower their sodium intake following study participation. The risk of the study procedures are low with placement of IV cannula as the most invasive study activity. Our previous protocols have demonstrated safety with respect to the imaging and kidney function tests.

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