Clinical Study Protocol

Drug Substance	Dapagliflozin
Study Code	DICE study
Edition Number	Version 5
Date	27-06-2015

Dapagliflozin on cholesterol metabolism in DM2: dissecting its effect on dyslipidemia by using stable isotope based cholesterol and glucose fluxes; a pilot study

Sponsor:

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

PROTOCOL SYNOPSIS

Dapagliflozin on cholesterol metabolism in DM2: dissecting its effect on dyslipidemia (DICE study)

Principal Investigator

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Study site(s) and number of subjects planned

AMC-VUmc Diabetes Center, 12 DM2 subjects to be included

Study period		**Phase of development**
Estimated date of first subject enrolled	Q4 2015	Phase III/IV
Estimated date of last subject enrolled	Q4 2017	Phase III/IV
Dataanalyses and modelling	Q1 2018	

Study design

Single arm intervention trial

Objectives

Primary Objective:	**Outcome Measure:**
effect of 5 weeks dapagliflozin 10mg on LDL cholesterol in patients with DM2	Change in LDL synthesis

Secondary Objective:	**Outcome Measure :**

Effect of 5 weeks dapagliflozin 10mg on and Triglyceride cholesterol fluxes	Change in VLDL secretion and clearance (as determined by $^{2}H_3$ Leucine enrichment) and relation to plasma CETP, PLTP and HDL subfractions Change in De novo lipogenesis (as determined by $^{2}H_2O$ deuterated 'heavy' water) oral 1,2,3,4-$^{13}C_{16}$ – palmitate to measure FFA remnant uptake.
Effect of 5 weeks dapagliflozin 10mg on insulin sensitivity and energy expenditure	Change in hepatic and peripheral insulin sensitivity (2 step Hyperinsulinemic normoglycemic clamp with $^{2}H_2$ enriched glucose:) and energy expenditure including carbohydrate oxidation and fatty acid oxidation rates in breathing air
Effect of 5 weeks dapagliflozin 10mg on liver fat content (MRI liver)	Change in liverfat MRI spectrum

Target subject population

Male or postmenopauzal female patients with type 2 diabetes BMI > 25 kg/m^2 and more than 12 weeks a stable dose of metformin treatment > 1500mg (HbA1C ≥6.5% - <8.5%) FPG<132 mmol/l, LDL cholesterol >2.5 mmol/l, willing to switch to rosuvastatin 10mg once daily for 4 weeks, and then receive 10 mg dapagliflozin once daily orally, for 5 weeks. Measurements (lipid and glucose fluxes) will be done after 4 weeks of rosuvastatin treatment (baseline) and after 5 weeks of dapagliflozin

Duration of treatment

4 weeks of crestor 10mg once daily treatment in all subjects (baseline) and after 5 weeks of dapagliflozin (n=12 DM2 subjects)

Investigational product, dosage and mode of administration

Crestor 10 mg once daily for in total 9 weeks orally

Dapagliflozin 10mg once daily for 5 weeks orally

Statistical methods

All data will be analysed using SPSS for Windows, version 20.0 (SPSS Inc. Chicago, Illinois, USA). Multivariate analysis and ANOVA for repeated measures will be used. Wilcoxon's signed-rank test will be used to compare results between the study groups. -Data will be expressed as median and range.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
AE	Adverse event
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IP	Investigational Product
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LSLV	Last Subject Last Visit
OAE	Other Significant Adverse Event
PGx	Pharmacogenetic research
PI	Principal Investigator
SAE	Serious adverse event
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background and rationale for conducting this study

There is a worldwide epidemic of obesity, which is a major risk factor for the development of common medical conditions such as type 2 diabetes (T2D), dyslipidemia and cardiovascular disease (CVD). Obesity and T2D are leading causes of morbidity and mortality in developed nations with an estimated cost exceeding 400 billion euro in the last 5 years and an estimated 250,000 deaths per year (1) The prevalence of obesity and T2D continues to rise: one out of three adults is expected to be diagnosed with T2D in 2050 (2) Current therapeutic regimens only minimally reduce T2D-related complications and lead to a shift from myocardial infarction to peripheral artery disease and kidney failure. Additionally, all-cause morbidity and mortality is still 10-fold higher in T2D patients compared to healthy subjects (3) This collectively represents a growing unmet clinical need.

In this regard, SGLT2 inhibitors improve glycemic control and are currently investigated in large cardiovascular outcome trials in patients with type 2 diabetes mellitus (4). It has been suggested that the beneficial cardiovascular effects are driven by both improved peripheral insulin sensivity (5,6) in conjunction with reduced bloodpressure and bodyweight in subjects with type 2 diabetes mellitus, the latter most likely driven by an osmotic (plasmacontraction) effect due to the increased glycosuria (7,8). However a potential disadvantage of all SGLT2 inhibitors might be the adverse effect seen on fasting lipid profiles including decreased HDL cholesterol and increased LDL-cholesterol and triglyceride plasma levels (9,10,11). In this regard ,there is a striking similarity with the observation of increased APOB synthesis due to plasma contraction in patients with nephrotic syndrome (12). Other mechanisms that might drive the observed dyslipidemic plasma cholesterol profile might include decreased hepatic LDL cholesterol clearance as well as deleterious effects of Cholesteryl ester transfer protein (CETP) resulting altered HDL cholesterol plasma levels. Epidemiological studies have unequivocally shown that high TG and low HDL-C represent strong independent risk factors of CVD. (13) Emerging evidence suggests that TG and HDL lipoproteins are strong interrelated biological parameters and linked to glucose homeostasis. Circulating triglycerides in part derive from VLDL-secretion from the liver as well as from Free Fatty Acid (FFA) uptake in the small intestine, a metabolic flux that is highly regulated by insulin (14). In obese insulin resistant subjects, insulin-mediated suppression of VLDL-synthesis and secretion is reduced resulting in high plasma TG and TG accumulation in the liver (15). Finally, SGLT2 is widely expressed throught the human body including in the small intestine (near GLP1 producing L cells) and in the liver (16). Thus suggesting that SGLT2 inhibitors can also affect glucose and lipid uptake in the gut.

We thus propose to further dissect the underlying mechanisms regarding dyslipidemic effects of SGLT2 inhibitors in relation to insulin sensivitity and energy metabolism in uncomplicated DM2 patients. This will be done using previously published stable isotope based techniques to study VLDL, LDL and HDL synthesis (15) as well as for determinination of hepatic and

peripheral insulin sensivitiy (5). All of these stable isotopes are GMP produced and analyses techniques have been previously used and published by our group (17, 18 and 19).

1.2 Rationale for study design, doses and control groups

Study Hypothesis and rationale

Dapagliflozin 10mg once daily for 5 weeks increases hepatic LDL and VLDL secretion driven by altered CETP in DM2 patients on stable statin therapy (10mg rosuvastatin once daily)

Study design:

Male or postmenopauzal female patients with type 2 diabetes (BMI > 25 kg/m2 and more than 12 weeks a stable daily dose of metformin treatment > 1500mg) with good glycemic control (HbA1C ≥6.5% - <8.5% and fasting plasma glucose <132 mmol/l) and on stable statin regiment and willing to switch to crestor 5 mg once daily during the study. After 4 weeks of rosuvastatin treatment (20), baseline measurements for glucose and lipid fluxes will be performed and therafter DM2 patients will be randomized to receive 10 mg dapagliflozin (n=12) orally with rosuvastatin 10mg once daily. Measurements (lipid and glucosefluxes) will be repeated after 5 weeks of dapagliflozin treatment.

Sample size

Previous studies have reported a 10 % increase in fasting plasma LDL plasma levels upon dapagliflozin 10mg once daily treatment in DM2 patients on statin therapy (20) As it can expected that metabolic effects of SGLT2 inhibition stabilize after 4 weeks (5,6) and lipid fluxes upon intervention are usually determined upon 4-5 weeks after start of intervention (21), we suggest to treat for 5 weeks. .A reduction was seen in 8 DM2 subjects of plasma LDL (from 3.1 ± 0.8 to 1.5 ± 0.4 mmol/l) on rosuvastatin treatment, that corresponded with a similar decrease in LDL- ApoB poolsize /synthesis (2.8 ± 0.4 to 1.5 ± 0.4 gram/day, see ref 22) . We thus expect 10% higher plasma LDL level (from 3.1 ± 0.8 to 1.7 ± 0.4 mmol/l) with concomitant less decrease in LDL- ApoB poolsize /synthesis (1.8 ± 0.4 gram/day) upon rosuvastatin combined with dapagliflozin 10mg treatment in DM2. Using single sided test (with alfa of 0.05 and 85% power) and using difference in LDL-apoB synthesis of 0.3 gram/day with SD of 0.4, the sample size needs to be 11 DM2 subjects, on dapagliflozin 10mg treatment. Taking a 10% patient dropout rate, we aim to include 12 DM2 subjects in total.

Benefit/risk and ethical assessment

Both rosuvastatin and dapagliflozin have been approved by FDA/EMEA and are widely prescribed. The effect of rosuvastatin alone on lipidfluxes in this group of DM2 patients has already been established (22) which thus allows us to study the mere effect of SGLT2 on

lipidfluxes in DM2 patients on stable statin therapy in a single arm trial. The use of stable isotope infusion is not associated with adverse events. Thus, the risk for patients to participate in this study is minimal.

1.3 Study Design

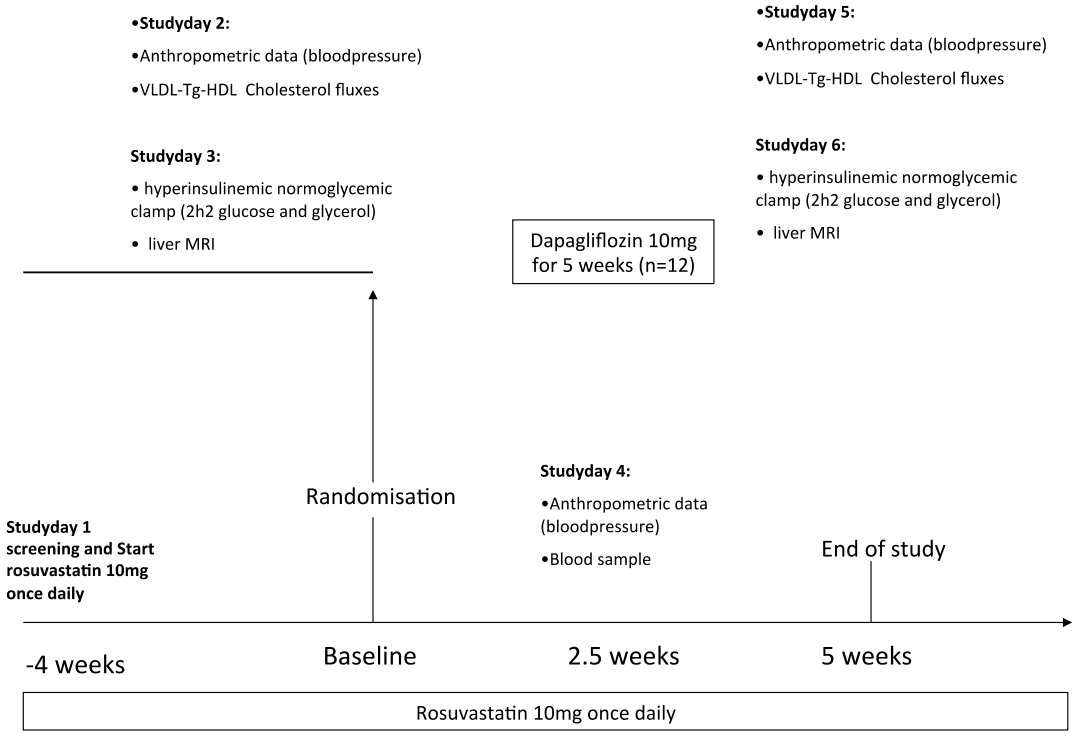

Figure 1

Study day 1: Assessment of cholesterol fluxes

VLDL, LDL, HDL kinetics*:* Subjects will drink $^{2}H_2O$ (2g per kg body weight), in the evening before the study day. First a blood sample will be drawn for determination of background enrichments. The subjects will sip the deuterated water between 18.00 and 22.00h. Thereafter, they are allowed to drink deuterated water (0.5% enriched) only to prevent dilution of the isotopic label.

The next day, subjects will be admitted to the metabolic unit at 7.30 AM after a 10 hour overnight fast. A catheter will be inserted into an antecubital vein for infusion of the stable isotope tracers. Another catheter will be inserted into a contralateral hand vein and kept in a thermo-regulated (60 C) plexiglas box for sampling of arterialized venous blood. Saline will be infused as NaCl 0.9% at a rate of 50 mL/h to keep the catheter patent. At T=0 (8.00 AM) a bolus of 7mg/kg of L[5.5.5-$^{2}H_3$]-leucine(99% enriched; Cambridge isotopes, Andover, USA) and simultaneously a [1,1,2,3,3-$^{2}H_5$] glycerol (99% enriched; Cambridge isotopes, Andover, USA) bolus of 500mg will be given. (23,24)

At T=0:02,0:04,0:06,0:08,0:10,0:12,0:15,0:20,0:30,0:45, 1,2,3, 4, 6, 8, 10 and 12h blood samples will be drawn for the determination of [2H3] leucine concentration in plasma. For the measurement of [2H3]-leucine and [2H5] glycerol in VLDL, LDL and HDL, blood samples will be drawn at T=-0.30, 0:30, 0:45, 1, 1:15, 1:30, 2, 2:30, 3, 4, 5, 6, 7, 8, 10, and 12h. In addition, at the above time-points blood samples will be drawn for measurement of plasma cholesterol, cholesteryl esters, FFA, apoB, apoAI and glycerol. At T=0, 4, 8 and 12h the particle composition and Apo-B mass of the VLDL and LDL fractions will be determined and the apoAI composition of HDL. See the study outline shown in figure 2.

At T=2.00 subjects will be served an oral fat load containing the dietary triglyceride tracer 1,2,3,4-13C16 – palmitate (1-2 grams). The meal will consist of bread, butter, cheese, low fat milk and tea or coffee (63 grams carbohydrates, 56 grams fat (P/S ratio 0.11) and 35.9 grams protein). Blood samples are drawn up to 8 hours after the meal (see figure 2).

During the test only deuterated water will be served ad libitum and the subjects will remain physically inactive. After the last blood sample at T=12h patients will be offered a meal. Time series data from enrichments of plasma leucine, leucine in apoB100 from VLDL and LDL and glycerol in VLDL and LDL are used as input to the kinetic model together with pool size measurements to simultaneously determine the kinetics of VLDL-TG and apo-B100 and LDL apoB and cholesterol. (23,24).

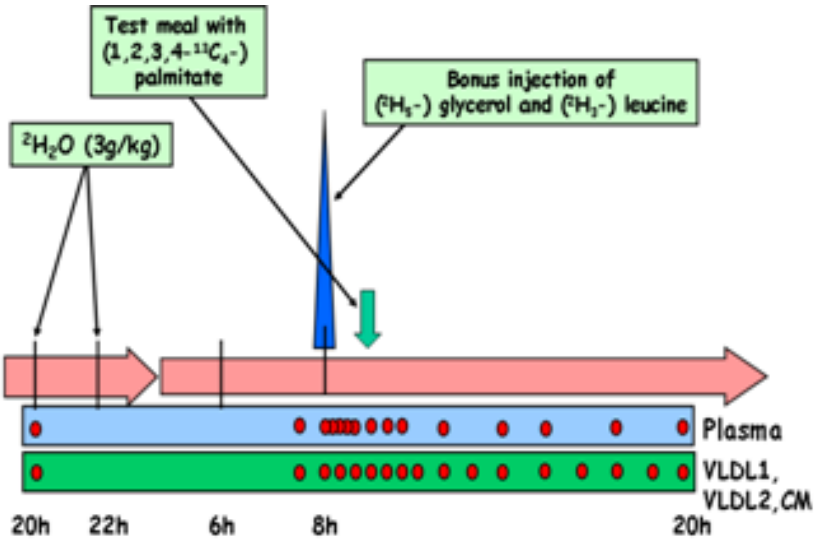

Figure 2: study design VLDL-HDL kinetics and visit 2 and 5.

Study day 2:

Assessment of glucose metabolism and lipolysis during a 2-step hyperinsulinemic euglycemic clamp

The clamp will be performed after an overnight fast. A catheter will be inserted into an antecubital vein for infusion of the stable isotope tracers (19). Another catheter will be inserted retrogradely into a contra lateral hand vein and kept in a thermo-regulated (60oC) plexiglas box for sampling of arterialized venous blood. Saline will be infused as NaCl 0.9% at a rate of 50 mL/h to keep the catheter patent. [6,6-2H2] glucose will be used as a glucose tracer (>99% enriched; Cambridge Isotopes, Andover, USA) to study total glucose production and insulin sensitivity. [1,1,2,3,3-2H5]-glycerol (>99% enriched; Cambridge Isotopes, Andover, USA) will be used to study glycerol turnover (as a measure of total triglyceride hydrolysis/lipolysis).

At T = -2, blood samples will be drawn for determination of the background enrichments and primed continuous infusions will be started: [6,6-2H2] glucose (prime =100 minutes of continuous infusion; continuous, 0.11 µmol/kg·min); [1,1,2,3,3-2H5]- glycerol (prime, 1.6 µmol/kg; continuous, 0.11 µmol/kg·min) and continued until the end of the study. At T=0, blood samples will be drawn for the determination of enrichments of glucose and glycerol 3 times with a 5 min interval. In addition, blood samples will be drawn for measurements of glucoregulatory hormones, free fatty acids (FFA), inflammatory markers and adipokines.

Resting energy expenditure (REE) will be measured during the final 20 minutes of these 2 hours and during the final 20 minutes of the study day. This will be done by indirect calorimetry. Oxygen consumption and CO2 production will be measured using a ventilated hood system. These measurements are then used to calculate REE and the respiratory quotient.At T=0:15, infusion of insulin (20mU/m2·min; Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, the Netherlands) and glucose 20% (to maintain a plasma glucose level of 5 mmol/L) will be started. [6,6-2H2] glucose will be added to the 20% glucose solution to achieve glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimizing changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. Plasma glucose levels will be measured every 10 minutes at the bedside. At T=2:15, 5 blood samples with an interval of 5 minutes will be drawn to determine glucose enrichments, glucoregulatory hormones, free fatty acids (FFA). At T=2:35 the insulin infusion will be increased (60mU/m2·min; Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, the Netherlands). Plasma glucose levels will be measured every 10 minutes at the bedside. At T=4:35, 5 blood samples with an interval of 5 minutes will be drawn for measurements of above mentioned parameters. At T= 4:55 insulin and the isotope tracers will be stopped and patients will be offered a bread meal. The glucose infusion will be tapered down to avoid hypoglycaemia.The stable glucose isotope ([6,6-2H2]glucose) will be obtained at the hospital pharmacy (AMC) and prepared for infusion by the coordinating investigator (19).

MRI based Quantification of intrahepatic triglycerides (IHTG)

IHTG will be quantified by 1H-MRS on study day 2 before the clamp procedure. 1H- MRS spectra will be acquired using a 1.0T Tesla open MRI (25). During the measurements, participants remain in the supine position within the MRI scanner. IHTG content will be obtained using single-voxel 1H-MRS, using a body array coil as the transmitter and phased surface coils as receivers. MRS measurements will be acquired during breath-hold, using single-voxel stimulated acquisition mode. Volumes of interest in the liver will be located away from major vascular structures and bile ducts. The water and fat resonance peaks, located at 4.65 and 1.3 ppm, will be integrated using jMRUI software and relative fat content will be expressed as the ratio of the fat peak area over the cumulative water and fat peak areas. Calculated peak areas of water and fat will be corrected for T2 relaxation (T2water, 34 ms, T2fat, 68 ms) and the percentage hepatic fat content will be calculated. The measurements will take approximately 30 minutes.

2. STUDY OBJECTIVES

Primary objective Objectives

Primary Objective:	**Outcome Measure:**
effect of 5 weeks dapagliflozin 10mg on LDL cholesterol in patients with DM2	Change in LDL synthesis

Secondary Objective:	**Outcome Measure :**
Effect of 5 weeks dapagliflozin 10mg on and Triglyceride cholesterol fluxes	Change in VLDL secretion and clearance (as determined by $^{2}H_3$ Leucine enrichment) and relation to plasma CETP, PLTP and HDL subfractions Change in De novo lipogenesis (as determined by $^{2}H_2O$ deuterated 'heavy' water) oral 1,2,3,4-$^{13}C_{16}$ – palmitate to measure FFA remnant uptake.
Effect of 5 weeks dapagliflozin 10mg on insulin sensitivity and energy expenditure	Change in hepatic and peripheral insulin sensitivity (2 step Hyperinsulinemic normoglycemic clamp with $^{2}H_2$ enriched glucose:) and energy expenditure including carbohydrate oxidation and fatty acid oxidation rates in breathing air
Effect of 5 weeks dapagliflozin 10mg on liver fat content (MRI liver)	Change in liverfat MRI spectrum

2.1 Safety objectives

Safety Objective: not applicable	**Outcome Measure :**

n/a	*n/a*

2.2 Exploratory objectives

Exploratory Objective:	**Outcome Measure :**
n/a	*n/a*

3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

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3.1 Inclusion criteria

Male or postmenopauzal female patients ;

Type 2 diabetes mellitus (HbA1C ≥6.5% - <8.5%)

At least 12 weeks a stable dose of metformin treatment > 1500mg FPG<132 mmol/l

LDL cholesterol >2.5 mmol/l,

Willing to switch used statin to crestor 10mg once daily for 9 weeks

3.2 Exclusion criteria

History of cardiovascular event

Smoking

Creatinin clearance < 60ml/min

Alcohol abuse (>4 units/day)

AST or ALT elevation (>2.5x upper limit)

3.3 Subject enrolment and randomization

All patients will receive dapagliflozin 10mg once daily.

3.4 Procedures for handling incorrectly enrolled or randomized subjects

Patients will be replaced by new patients until all 12 DM2 patients have completed the trial

3.5 Methods for assigning treatment groups

n/a

3.6 Methods for ensuring blinding

n/a

3.7 Methods for unblinding

n/a

3.8 Restrictions

None

3.9 Discontinuation of investigational product

3.9.1 Procedures for discontinuation of a subject from investigational product

Patients will be replaced by new patients until all 12 DM2 patients have completed the trial.Dropout patients will be followed up until 3 months after cessation of the trial.

3.10 Criteria for withdrawal

none

3.10.1 Screen failures

When patients do not match inclusion criteria, they will not be enrolled in the trial.

3.10.2 Withdrawal of the informed consent

Patients will be replaced by new patients until all 12 DM2 patients have completed the trial.Dropout patients will be followed up until 3 months after cessation of the trial.

3.11 Discontinuation of the study

Dropout patients will be followed up until 3 months after cessation of the trial.

4. STUDY PLAN AND TIMING OF PROCEDURES

Table 1 Study Plan DICE study detailing the procedures

Visit Visit window Days –28 to 0 for Visit 1 7 ±1 day for Visit 2& 3 7 ±1 day for Visits 4 7 ±1 day for Visit 5&6	**1** **Screening and start rosuvastatin**	**2**	**3**	**4**	**5**	**6**		For details see Protocol Section
Week		**0**	**0**	**2.5**	**5**	**5**		
Day	**-**							
Written informed consent *(including tissue samples)*	X							
Demographics	X							
Physical examination, height and weight	X			X		X		
Medical/surgical history/ECG	X							
Inclusion/exclusion criteria	X							
Fluxes and MRI		X	X		X	X		
Vital signs	X	X	X	X	X	X		
Start study treatment			X					
Treatment dispensed/returned	X	X					X	
Concomitant medication	X	X	X	X	X	X	X	

Visit	**1**	**2**	**3**	**4**	**5**	**6**	For details see Protocol Section
Visit window Days –28 to 0 for Visit 1 7 ±1 day for Visit 2& 3 7 ±1 day for Visits 4 7 ±1 day for Visit 5&6	**Screening and start rosuvastatin**						
Week		**0**	**0**	**2.5**	**5**	**5**	
Day	**-**						
Adverse event review (AEs and SAEs)		X	X	X	X	X	
Blood samples for haematology and clinical chemistry	X	X	X	X	X	X	
24h Urinalysis		X	X		X	X	

4.1 Enrolment/screening period

Q3 2015

4.2 Treatment period

5 weeks

4.3 Follow-up period

none

5. STUDY ASSESSMENTS

5.1 Efficacy assessments

Change in lipolysis using plasma VLDL-TG clearance (as determined by $^{2}H_5$ glycerol enrichment)

Change in VLDL-TG secretion and clearance (as determined by $^{2}H_3$ Leucine enrichment) in relation to plasma CETP, PLTP and HDL subfractions

Change in cholesterol mobility (13C cholesterol enrichment in LDL and HDL)

Change in De novo lipogenesis (as determined by $^{2}H_2O$ deuterated 'heavy' water)

oral 1,2,3,4-$^{13}C_{16}$ – palmitate to measure FFA remnant uptake.

5.2 Safety assessments

5.2.1 Laboratory safety assessments

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
B-Haemoglobin (Hb)	S/P-Creatinine
B-Leukocyte count	S/P-Bilirubin, total
B-Leukocyte differential count (absolute count)	S/P-Alkaline phosphatise (ALP)
B-Platelet count	S/P-Aspartate transaminase (AST)
	S/P-Alanine transaminase (ALT)
Urinalysis (dipstick)	S/P-Albumin
U-Hb/Erythrocytes/Blood	S/P-Potassium
U-Protein/Albumin	S/P-Calcium, total
U-Glucose	S/P-Sodium

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5.2.2 Physical examination

Regular

5.2.3 ECG

5.2.3.1 Resting 12-lead ECG

Done on screeningsvisit

5.2.4 Vital signs

Will be taken according to AMC Vascular Medicine SOP

5.2.4.1 Pulse and blood pressure

Will be taken according to AMC Vascular Medicine SOP

5.2.4.2 Body temperature

Will not be taken routinely

5.2.5 Other safety assessments

n/a

5.3 Other assessments

5.3.1 Patient reported outcomes

Not used

5.4 Pharmacokinetics

5.4.1 Collection of samples

n/a

5.4.2 Determination of drug concentration

n/a

5.4.3 Storage and destruction of pharmacokinetic samples

n/a

5.5 Pharmacodynamics

5.5.1 Collection of samples

n/a

5.5.2 Storage, re-use and destruction of pharmacodynamic samples

n/a

5.6 Pharmacogenetics

5.6.1 Collection of pharmacogenetic samples

n/a

5.6.2 Storage, re-use and destruction of pharmacogenetic samples

n/a

5.7 Biomarker analysis

5.7.1 Storage, re-use and destruction of biological samples

Samples will be stored at -80C at AMC and used for analyses. Upon 5 years after completion of the study, samples will be destroyed.

5.7.2 Labelling and shipment of biological samples

Samples will be labelled according to entry number; upon shipment, they will be only be shipped by Worldcourier

5.7.3 Chain of custody of biological samples

Samples will be owned by AMC hospital

5.7.4 Withdrawal of Informed Consent for donated biological samples

Samples will be destroyed upon retraction of informed consent.

6. SAFETY REPORTING AND MEDICAL MANAGEMENT

6.1 Definition of adverse events

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational drug or deterioraton of a pre-existing medical condition. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

6.2 Recording of adverse events

Will be recorded in CRF

6.2.1 Time period for collection of adverse events

During 9 weeks of being in the study

6.2.2 Follow-up of unresolved adverse events

Patients will be followup until 3 months after end of trial visit

6.2.3 Variables

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational drug. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. The following variables should be collected for each AE;

- *AE (verbatim)*
- *The date and time when the AE started and stopped*
- *Whether the AE is serious or not*
- *Investigator causality rating against the Investigational Product (yes or no)*
- *Action taken with regard to investigational product*
- *AE caused subject's withdrawal from study (yes or no)*

- *Outcome.*
- *In addition, the following variables should be collected for SAEs:*
- *Date AE met criteria for serious AE*
- *Date Investigator became aware of serious AE*
- *AE is serious due to...*
- *Date of hospitalisation*
- *Date of discharge*
- *Probable cause of death*
- *Date of death*
- *Autopsy performed*
- *Causality assessment in relation to Study procedure(s)*
- *Description of AE*
- *Causality to assessment in relation to study procedure(s)*
- *Causality assessment in relation to other medication*
- *(Causality assessment in relation to additional study drug)*
-

6.2.4 Causality assessment

6.2.5 Causality will be assessed by study physician and Principle Investigator.

6.2.6 Disease progression

Not applicable in such a short timeframe

6.3 Reporting of serious adverse events

A serious adverse event is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above any untoward medical occurrence, effect or deterioration of a pre-existing medical condition that at any dose:

- results in death;
- is life-threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section. In accordance to section 10, subsection 1, of the WMO, the investigator will inform immediately the subjects, the reviewing accredited MEC and concurrently AstraZeneca if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited MEC/IRB, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

The investigator will report the SAE's through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 15 days after the investigator has first knowledge of the serious adverse reaction and concurrently to AZ.

SAE's that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report Also, a concurrently SAE report and accompanying cover page will be send by e-mail (AEMailboxClinicalTrialTCS@AstraZeneca.com) to AstraZeneca TCS Data Entry Site.

On behalf of the sponsor, the PI will submit once a year an Annual Safety Report to the accredited MEC and competent authority.

6.4 Overdose

Patients will be admitted to AMC hospital (known symptoms:

6.5 Pregnancy

Pregnancy test will be performed at inclusion, since all females will be postmenopausal no checkup will be done thereafter

6.5.1 Maternal exposure

Pregnancy test will be performed at inclusion, since all females will be postmenopausal no checkup will be done thereafter

6.5.2 Paternal exposure

Will not be studied

6.6 Management of toxicities <<Dose Reductions>>

Will not be studied

6.7 Study governance and oversight

6.7.1 Steering Committee

Will not be installed

6.7.2 Data Monitoring Committee

Study will be monitored by Clinical Research Unit of the AMC (head: JJ van Dalen)

6.7.3 Scientific Advisory Committee

Will not be installed

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Dapagliflozin	10mg 1dd	Astrazeneca

Rosuvastatin	10mg 1dd	Astrazeneca

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7.2 Dose and treatment regimens

10mg dapagliflozin once daily for 5 weeks ; rosuvastatin 10mg once daily for 9 weeks. Both will be supplied by AstraZeneca Netherlands, free of charge.

7.3 Labelling

Will be done by AMC clinical pharmacy

7.4 Storage

Will be done at AMC clinical pharmacy

7.5 Compliance

Will be checked by physician and nurse countil returned pills

7.6 Accountability

Will be done by AMC clinical pharmacy

7.7 Concomitant and other treatments

Restricted Medication/Class of drug:	**Usage:**
insulin	

Prohibited Medication/Class of drug:	
insulin	

Rescue/Supportive Medication/Class of drug:	**Usage:**
n/a	

7.7.1 Other concomitant treatment

7.8 Post Study Access to Study Treatment

Both rosuvastatin and dapagliflozin can be prescribed by physicians in the Netherlands

8. STATISTICAL ANALYSES

8.1 Statistical considerations

8.2 Sample size estimate

Previous studies have reported a 10 % increase in fasting plasma LDL plasma levels upon dapagliflozin 10mg once daily treatment in DM2 patients on statin therapy (20) As it can expected that metabolic effects of SGLT2 inhibition stabilize after 4 weeks (5,6) and lipid fluxes upon intervention are usually determined upon 4-5 weeks after start of intervention (21), we suggest to treat for 5 weeks. .A reduction was seen in 8 DM2 subjects of plasma LDL (from 3.1 ± 0.8 to 1.5 ± 0.4 mmol/l) on rosuvastatin treatment, that corresponded with a similar decrease in LDL- ApoB poolsize /synthesis (2.8 ± 0.4 to 1.5 ± 0.4 gram/day, see ref 22) . We thus expect 10% higher plasma LDL level (from 3.1 ± 0.8 to 1.7 ± 0.4 mmol/l) with concomitant less decrease in LDL- ApoB poolsize /synthesis (1.8 ± 0.4 gram/day) upon rosuvastatin combined with dapagliflozin 10mg treatment in DM2. Using single sided test (with alfa of 0.05 and 85% power) and using difference in LDL-apoB synthesis of 0.3 gram/day with SD of 0.4, the sample size needs to be 11 DM2 subjects, on dapagliflozin 10mg treatment. Taking a 10% patient dropout rate, we aim to include 12 DM2 subjects in total.

8.3 Definitions of analysis sets

Before and after dapagliflozin treatment

8.3.1 Efficacy analysis set

Effect dapagliflozin on HDL catabolic fractional rate and plasma CETP levels

Effect dapagliflozin on triglyceride/VLDL synthesis rate and liver fat MRI signal

Effect dapagliflozin on hepatic and peripheral insulin sensitivity

8.3.2 Safety analysis set

n/a

8.3.3 PK analysis set

n/a

8.3.4 PRO analysis set

n/a

8.4 Outcome measures for analyses

8.5 Methods for statistical analyses

8.5.1 Analysis of the primary variable(s)

All data will be analysed using SPSS for Windows, version 20.0 (SPSS Inc. Chicago, Illinois, USA). Multivariate analysis and ANOVA for repeated measures will be used. Wilcoxon's signed-rank test will be used to compare results between the study groups. Data will be expressed as median and range. Spearman's rank test will be used to calculate correlations.

8.5.2 Analysis of the secondary variable(s)

All data will be analysed using SPSS for Windows, version 20.0 (SPSS Inc. Chicago, Illinois, USA). Multivariate analysis and ANOVA for repeated measures will be used. Wilcoxon's signed-rank test will be used to compare results between the study groups. Data will be expressed as median and range. Spearman's rank test will be used to calculate correlations.

8.5.3 Subgroup analysis (if applicable)

n/a

8.5.4 Interim analysis

N/a

8.5.5 Sensitivity analysis (if applicable)

n/a

8.5.6 Exploratory analysis (if applicable))

n/a

9. STUDY AND DATA MANAGEMENT

9.1 Training of study site personnel

Personall will be trained according to NFU/BROK regulations.

9.2 Monitoring of the study

Will be done by Clinical Research Unit of AMC

9.2.1 Source data

Data will be stored in a SPSS based database, all source data can be verified by monitor upon request.

9.2.2 Study agreements

AstraZeneca and AMC will draft a contract for this project. The sponsor will have no say in the analyses or publication of the data.

9.2.3 Archiving of study documents

Source documents will be stored at AMC archive

9.3 Study timetable and end of study

Study will run for 2-2.5 years with another 3-6 months for data analyses/modelling

9.4 Data management

Serious Adverse Event (SAE) Reconciliation

All SAE will be reported to the accredited AMC IRB/MEC that approved the protocol, according to the requirements of the AMC IRB/MEC

Data Management of genotype data

n/a

Data associated with human biological samples

According to local AMC rules

Management of external data

10. ACCORDING TO LOCAL AMC RULESETHICAL AND REGULATORY REQUIREMENTS

10.1 Ethical conduct of the study

The study will be approved by local AMC IRB (MEC)

10.2 Subject data protection

All data will be anonymous (patients will receive entry number upon enrolment in the study)

10.3 Ethics and regulatory review

See above

10.4 Informed consent

The Principal Investigator(s) at each centre will:

Delegate informed consent procedure to study physicians

10.5 Changes to the protocol and informed consent form

IRB/MEC will be informed upon changes to procotol

10.6 Budget aanpassen

Salary MD PhD student 27 months		125k
Patient participation+travel fee	15k	
Stable isotopes (GMP produced)		70k
Biochemistry + MassSpec analyses		10k:
15% AMC overhead incl AMC pharmacy		33k +
total Budget		**253.000 euro**

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