Statistical Analysis Plan

Study Code	D1690C00047
Edition Number	2.0
Date	18th December 2019

DAPAMAAST: A Double-blind, Randomized, Phase IV, Mechanistic, Placebo-controlled, Cross-over, Single-center Study to Evaluate the Effects of 5 Weeks Dapagliflozin Treatment on Insulin Sensitivity in Skeletal Muscle in Type 2 Diabetes Mellitus Patients

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Study Statistician [redacted] 20-Dec-2019
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Global Product Statistician [redacted]

Daniel Reyner

21 Dec 2019

Date

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IQVIA Statistician [redacted] 20DEC2019
Date

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LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
AC	Acetylcarnitine
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis Of Variance
AST	Aspartate Aminotransferase
AUC	Area under the curve
BH	Benjamini-Hochberg
BMI	Body Mass Index
cGDR	corrected Glucose Disposal Rate
CRA	Clinical Research Associate
CRAT	Carnitine Acetyltransferase
CS	Citrate Synthase
DAE	Discontinuation of Investigational Product due to Adverse Event
DEXA	Dual-Energy X-ray Absorptiometry
DKA	Diabetic Ketoacidosis
DPPIV	Dipeptidyl peptidase IV
EAS	Evaluable Analysis Set
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EGP	Endogenous Glucose Production
EHC	Euglycemic Hyperinsulinemic Clamp
EOT	End of treatment
FA	Fatty acid
FDR	False discovery rate
FGF21	Fibroblast Growth Factor 21
GDR or RD	Glucose Disposal Rate or Rate of Disposal
Hb	Hemoglobin
HbA1c	Hemoglobin A1c
HL	Hy's Law

Abbreviation or special term	Explanation
hsCRP	High-sensitivity C-Reactive Protein
IHLC	Intrahepatic lipid content
IMCL	Intramyocellular lipids
IP	Investigational Product
IPD	Important protocol deviation
ITT	Intent-to-Treat
LSM	Least Square Mean
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NEFA	Non-esterified fatty acids
NOGD	Non-oxidative glucose disposal
PCr	Phosphocreatine recovery
PD	Protocol deviation
PHL	Potential Hy's Law
RER	Respiratory Exchange Ratio
RQ	Respiratory Quotient
SAE	Serious Adverse Event
SD	Standard Deviation
SE	Standard Error
T2DM	Type 2 Diabetes Mellitus
VO_2max	Maximal Oxygen consumption
WHO	World Health Organisation

AMENDMENT HISTORY

Date	Brief description of change
	N/A
20th February 2019	Section 1.2 – Text added for clarity on defining drop-outs. Section 3.3.1 - Adverse event windowing updated to remove 5-10 days follow-up window due to site collection dates being longer than protocol outlined. Section 4.2.2 - Prior medication definition updated from stop date to start date. Section 4.2.3 – Compliance reporting clarified to specify missing calculations in treatment period and overall if a return bottle is missing. Section 4.2.4.1 – Detail added on residuals being assessed for normality during model checking. Clarification on response variable of change between Dapagliflozin and Placebo being modelled for the linear regression model. Section 4.2.5.1 – Imputation rules for missing intensity, causality and seriousness updated in line with the Dapa Mech program. Appendix B updated with amendment to efficacy endpoints collected.
21st October 2019	Section 2.1.3 – Evaluable analysis set updated to clarify subject exclusions for procedure specific PDs will lead to exclusion of the subject from both periods for an endpoint if at least one period is missing data. Use of planned treatment instead of treatment received for outputs. Section 3.1.3 – Definition for study day calculation separated to distinguish between on or after first dose of IP and prior to first dose of IP. Section 3.2.1 – Text added to clarify discrepancy in terminology used in the protocol for the primary efficacy endpoint and data to be received from the site. Section 3.3.1 – Removed off-treatment definition from AEs and reference to combined period. Section 3.3.6.1 and 4.2.1.2 – Reference to addition of baseline metformin dose categorization in outputs and categories to be summarized. Section 4.2.1.1, 4.2.1.2, 4.2.2 and 4.2.3 – Update to analysis populations used for outputs and reference to those used in shells already, stated in the relevant sections. Section 4.2.1.2 – Clarity added for medical and surgical history tables to include specific medical history in them as well. Section 4.2.4.1 – Model assumption checks reduced due to update in efficacy approach removing treatment-, period- and sequence- heterogeneous analyses from sensitivity. Section 4.2.4.2 – Additional text for vital signs outputs to model change from baseline separately for day's 15 and 30. Multiplicity sort order clarified for p-values feeding into Benjamini-Hochberg analysis. Section 4.2.5.1 – Reference to adverse events clarified to state only serious adverse events (SAEs), AEs causing discontinuation of IP (DAEs) and potential DKA events will be presented for in outputs. Appendix B updated with amendment to efficacy endpoints collected.

Date	Brief description of change
20th November 2019	Section 1 – Protocol version updated from 4 to 5. Section 1.2 – Text amended to reference end of treatment visits only in determining drop outs needing replacement. Section 2.1.3 – EAS MRS Lactate subset added to list. Details about a separate populations report mentioned. Change from planned treatment to treatment received. Section 3.2.2 – Exploratory endpoint list updated and derivation for urine chamber parameters daytime and night-time calculations added over 24 hours. Section 3.3.1.1 – Guidance document for DKA reporting referenced. Section 4.2.1.2 – Updated to outline demographic and baseline characteristic outputs to be summarized by Randomized analysis set as well as EAS. Section 4.2.2 – Prior medications changed from start date less than or equal to first day of IP to just less than first day of IP. Section 4.2.3 – Clarification added to state extent of exposure is calculated for each treatment period using the same formula. Section 4.2.4.1 – Within-subject difference for linear regression model updated. Section 4.2.4.2 – Information on lactate parameter analysis added, outlining model to be performed as well as summary statistics and boxplot to be presented. Area under the curve for calculating the trapezoidal method on chamber blood parameters incorporated, including how to handle missing samples. Section 4.2.5.1 – Reference to AE counts being presented, removed. Clarification around DKA events to be listed. Section 6 – Text on AUC as a change from protocol outlined. Appendix B – Additional endpoints added for lactate, glucose urine loss (24h), protein oxidation (24h) and creatinine daytime (12h), night-time (12h) and (24h).
3rd December 2019	Section 2.1.3 – Text added outlining update in process by the site determining invalid results not to be used for analysis. Section 2.2.1 – Process of programmed deviations used for monitoring in the study clarified. Section 3.1.2 – Derivation for within-subject difference added. Section 3.1.4 – Wording update to explain clearly EOT derivation for each subject. Section 3.2.1 and 4.2.4.1 – Primary endpoint updated to align with Appendix B. Section 3.3 – Clarified pulse will not be assessed as efficacy as per CSP. Double blind changed to double-blind throughout for consistency.

Date	Brief description of change
11th December 2019	Section 3.2.2 - Change in daytime and night-time calculations for laboratory urine assessments. Section 4.2.1.2 – Baseline characteristic VO_2 max/lean body mass updated to be divided by total body mass. Appendix B – reference to 12h for laboratory urine assessments removed. Reference to SMR day 1 and day 2 removed. Addition of first acetylcarnitine (AC)/after exercise.

1. STUDY DETAILS

This is the statistical analysis plan (SAP) for study D1690C00047. The SAP describes the statistical analyses specified in Version 5 of the clinical study protocol (CSP) in more detail; any changes with regards to what is already specified in the CSP will be described in Section 6.

1.1 Study objectives

1.1.1 Primary objective

To investigate if dapagliflozin improves skeletal muscle insulin sensitivity expressed as corrected glucose disposal rate (cGDR) in comparison with placebo after 5-week double-blind treatment. Insulin sensitivity will be determined using a 2-step euglycemic hyperinsulinemic clamp (EHC) procedure.

1.1.2 Safety objectives

To evaluate the safety and tolerability of dapagliflozin by assessment of discontinuation of Investigational Product (IP) due to adverse event (DAE)/serious adverse events (SAE), including laboratory values and clinically significant findings after 5-week double-blind treatment.

1.1.3 Exploratory objectives

1. To investigate if dapagliflozin changes Endogenous Glucose Production (EGP) in comparison with placebo after 5-weeks of double-blind treatment.

2. To investigate if dapagliflozin improves metabolic flexibility as compared to placebo, determined by the change in respiratory exchange ratio (RER) from fasted state to insulin stimulated state during EHC after 5-week double-blind treatment.

3. To investigate if dapagliflozin changes RER and energy expenditure as well as plasma metabolites such as beta-hydroxybutyrate and glucose as compared to placebo, before and after meals in the metabolic chamber after 5-week double-blind treatment.

4. To investigate if dapagliflozin improves maximal capacity to form acetylcarnitine following exercise, and *Carnitine Acetyl Transferase (CRAT) and citrate synthase activities in muscle biopsy, as compared to placebo after 5-week double-blind treatment.

5. To investigate if dapagliflozin improves in vivo mitochondrial function as measured by phosphocreatine recovery following exercise, as compared to placebo after 5-week double-blind treatment.

6. To investigate if dapagliflozin improves ex vivo mitochondrial function in permeabilized muscle fibers using high resolution respirometry, as compared to placebo after 5-week double-blind treatment.

7. To investigate if dapagliflozin changes body composition, skeletal muscle and liver fat content, as compared to placebo after 5-week double-blind treatment.

8. To investigate if dapagliflozin changes blood biomarkers such as glucagon, insulin and FGF21 in comparison with placebo after 5-weeks of double-blind treatment.

9. *To investigate if dapagliflozin changes expression of mRNA and/or proteins involved in metabolic regulation in muscle tissue in comparison with placebo after 5-weeks of double-blind treatment.

10. To investigate if dapagliflozin changes body weight, body mass index (BMI) and systolic and diastolic blood pressure in comparison with placebo after 5-weeks of double-blind treatment.

A full list of exploratory endpoints associated with the objectives can be found in Appendix B.

*Note: These exploratory end-points are not planned to be reported in the CSR since these analyses are exploratory and will be done by an external partner and/or will be done after all other analyses are performed. Further descriptions of analyses associated with these endpoints are not covered in this SAP.

1.2 Study design

This is a double-blind, randomized, mechanistic, placebo-controlled, cross-over study, to evaluate the effect of 5-weeks dapagliflozin treatment in Type 2 Diabetes Mellitus (T2DM) in subjects either on a stable dose of metformin and/or a dipeptidyl peptidase IV (DPPIV) inhibitor for at least the last 3 months or are drug naïve.

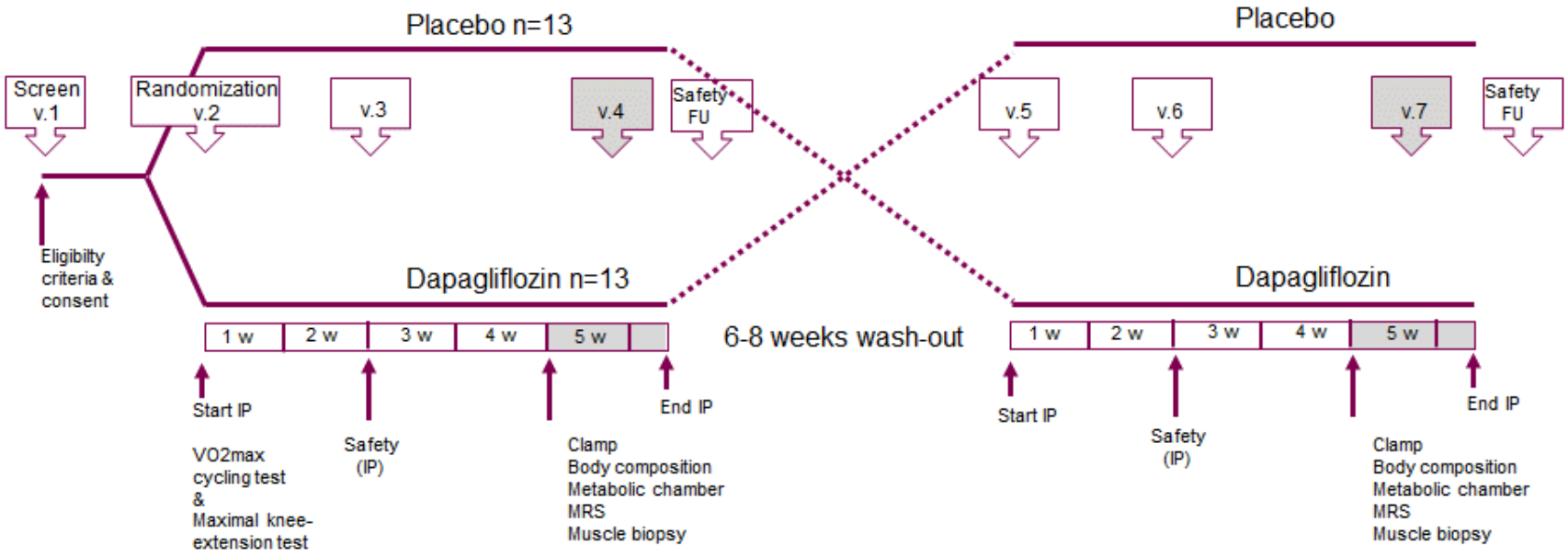
A total of 26 subjects are planned to be randomized to have at least 22 completers in two equal sized treatment sequences. In case of more than 4 drop-outs, not attending both end of treatment visits (visit 4 and visit 7), another 4 subjects will be randomized and if this occurs, a total of 30 subjects will be randomized.

Subjects will participate in two treatment periods. In period 1 subjects will receive either dapagliflozin 10 mg or matching placebo for a maximum of 40 days based on randomization sequence. For period 2, subjects that received 10 mg dapagliflozin in the first treatment period will receive matching placebo in the second treatment period and subjects who received placebo in the first treatment will receive 10 mg dapagliflozin in the second treatment period, for a maximum of 40 days.

All potentially eligible subjects will be asked to provide informed consent and thereafter be screened to assess eligibility criteria at Screening (Visit 1). Subjects who meet eligibility

criteria will be randomized to one of two treatment sequences. The first 5-week treatment period will involve the first dose of dapagliflozin/placebo administered via tablet at Week 0 (this is considered to be Day 1, for the purpose of analysis). Subjects will continue dapagliflozin or placebo until end of study period, Day 29±3 (+ 6–8 days for final assessments). After the last day of the end of treatment (EOT) visit, treatment with dapagliflozin or placebo will be discontinued and subjects will enter a 6–8 weeks wash-out period. A safety follow- up will be performed about 1 week (5-10 days) after the EOT visit. After completion of the 6–8-week wash-out period, the cross over will take place and subjects having received dapagliflozin will receive the placebo and subjects having received placebo will receive dapagliflozin over a 5-week treatment period. A safety follow-up will again be performed 5–10 days post-last dose. A graphical view of the study is shown in Figure 1. For a detailed overview of study design and the performed assessments, see CSP Table 4.

Figure 1 Study flow chart



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2. ANALYSIS SETS

2.1 Definition of analysis sets

2.1.1 Enrolled analysis set

This analysis set consists of all subjects who sign the informed consent form and will be used for the reporting of disposition and screening failures.

2.1.2 Randomized analysis set

The Randomized analysis set will consist of all randomized subjects. This is also known as the intent to treat (ITT) population. In analyses using the Randomized analysis set, subjects will be represented using the treatment to which they were randomized (even if the treatment they received is different). This analysis set will be used for reporting important protocol deviations (IPD), analysis sets and disallowed medications.

2.1.3 Evaluable analysis sets

The Evaluable analysis set (EAS) will be the primary analysis set for efficacy and is a subset of the Randomized analysis set, consisting of subjects who received at least 1 dose of IP. This analysis set will exclude subjects with IPDs that may greatly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. This is also known as the Per-Protocol (PP) population.

To evaluate the primary and exploratory endpoints, relevant protocol deviations (PDs) that are procedure specific will also be assessed. These PDs may lead to subjects being excluded from particular analyses (but not from all of them). Examples of such PDs are insufficient quality of a sample, or a subject failing to fulfil a procedure-specific requirement such as fasting for 6 hours. To evaluate each endpoint effectively the following subsets of the Evaluable analysis set are to be utilised:

- EAS Clamp
- EAS Chamber
- EAS Biomarkers
- EAS Indirect Calorimetry
- EAS MRS Acetylcarnitine
- EAS MRS Liver Lipid (IHLC)

- EAS MRS Muscle Lipid (IMCL)
- EAS MRS Mitochondrial (PCr recovery rate)
- EAS MRS Lactate
- EAS DEXA (body composition)
- EAS Muscle Biopsy (high resolution respirometry using muscle fibers)

All values for the efficacy endpoints will be evaluated for validity prior to judgement on PDs, where invalid results are noted and will not be used in analysis. Subjects for whom both the data from the first and second period is judged to be unreliable due to PDs will be excluded from the data set corresponding to the measurement in question. Subjects for whom the data from only one endpoint is judged to be unreliable for a procedure will be included in the population total for the EAS subset, but the subject will be excluded from the analyses for the endpoint. All decisions to exclude data from the Evaluable analysis sets will be made prior to the database lock of the study and treatment unblinding and will be documented in a separate populations report.

Subjects' data will be represented using the actual treatment received.

2.1.4 Safety analysis set

The Safety analysis set will consist of all subjects who received at least one dose of study drug, with subjects being analyzed and presented by treatment received, rather than as randomized.

2.2 Violations and deviations

2.2.1 Protocol deviation monitoring

During study conduct, protocol deviations will be closely monitored and identified from two sources:

- Manually entered protocol deviations identified by Clinical Research Associate (CRA) during the study conduct.

- Statistically programmed protocol deviations. These are deviation checks will be generated by execution of programs written using the predefined deviator descriptions in the SAP and protocol. Any potential PDs will be fed back to the study team to be manually entered into IMPACT prior to unblinding.

2.2.2 Protocol deviation reporting

The description of different types of PDs, as well as the classification of the PDs will be provided in a separate protocol deviation document outside of the SAP. Briefly, the PDs include, but are not limited to:

- Subjects who do not meet the inclusion criteria

- Subjects who meet any of the exclusion criteria
- Subjects who use one or more prohibited medication
- Subjects who received the incorrect investigational treatment or dose at any time
- Protocol-required procedures not adhered to
- Incorrectly unblinded to treatment during study participation
- General study conduct

All reported PDs will be reviewed, and IPDs will be identified and documented by the AZ study physician and statisticians prior to unblinding of the data. Protocol deviations that the study team considers to be important will be tabulated or listed.

The summaries of IPDs will be done by treatment sequence and overall. Separate listings of all subjects with important protocol deviations will also be produced. All data removed due to protocol deviations will also be listed.

3. PRIMARY AND EXPLORATORY VARIABLES

3.1 General Definitions

3.1.1 Definition of baseline

For safety and efficacy endpoints, where applicable, the last measurement prior to first dose of study treatment at Visits 2 and 5 will serve as the baseline measurements for the two treatment periods, omitting unscheduled visits.

3.1.2 Absolute change from baseline

Absolute change from baseline outcome variables is computed separately for each treatment period as

(post-treatment value – baseline value).

If either the post-treatment value or the baseline value is missing, then the absolute change from baseline value will also be set to missing.

A similar derivation will be used for the within-subject difference, regardless of treatment sequence, calculated as

(dapagliflozin – placebo)

3.1.3 Study day

Study day will be defined relative to the first dose of IP in the given treatment period and will be calculated as

If on or after the first dose of IP in Periods 1 or 2: (date of assessment/event – date of first dose of IP in the given treatment period) + 1.

If before the first dose of IP in Period 1: (date of assessment/event – date of first dose of IP in the given treatment period).

Day 1 will be reset for each treatment period, with washout and follow-up periods being calculated relative to the previous treatment period. Treatment period will be indicated where study day is presented.

3.1.4 Definition of end of treatment

End of treatment (EOT) assessments are defined as assessments captured at Visits 4 or 7. For overall EOT assessments for a subject this will be the last of Visits 4 and 7 assessments regardless of treatment sequence. Where either visit is missing for an efficacy endpoint, the EOT value for a subject will not be imputed. For evaluation of changes in safety endpoints, if the scheduled EOT within a study period for a subject is not performed, not evaluable or is missing, but an unscheduled assessment is available, then the last unscheduled assessment in the period will be used.

3.2 Calculation or derivation of efficacy variables

3.2.1 Primary efficacy endpoint

The primary endpoint is the skeletal muscle insulin sensitivity measured as cGDR, which will now be referred to as delta RD (basal vs high insulin), using a 2-step EHC procedure after 5 weeks of treatment. For the primary analysis, the response variable is the delta RD (basal vs high insulin) measured at the end of treatment period 1 and 2, captured at Visits 4 and 7 respectively. Delta RD (basal vs high insulin) will be calculated and provided by the site.

3.2.2 Exploratory efficacy endpoints

Comparison of dapagliflozin versus placebo after 5 weeks treatment will be given for the following:

1. Change of Endogenous Glucose Production (EGP) during EHC.

2. Metabolic flexibility, as determined by the RER using indirect calorimetry from fasted state to insulin (high insulin) stimulated state during EHC.

3. RER and energy expenditure as well as plasma metabolites such as betahydroxybutyrate and glucose before and after meals, as measured in metabolic chambers.

4. Maximal capacity to form acetylcarnitine following exercise as measured using ^{1}H-MRS, and *CRAT and CS activities from muscle biopsy.

5. In vivo mitochondrial function as measured post-exercise by phosphocreatine recovery using ^{31}P-MRS.

6. Ex vivo mitochondrial function in permeabilized muscle fibers using high resolution respirometry (muscle biopsy).

7. Body composition as measured by DEXA, and skeletal muscle and liver fat content as measured by ^{1}H-MRS.

8. Change of blood biomarkers such as glucagon, insulin and FGF21.

9. *Change of expression of mRNA and/or proteins involved in metabolic regulation in muscle tissue.

10. Change from baseline in body weight, BMI and systolic and diastolic blood pressure.

*Note: These exploratory end-points are not planned to be reported in the CSR since these analyses will be done by an external partner and/or will be done after all other analyses are performed. Further descriptions of analyses associated with these endpoints are not covered in this SAP.

A comprehensive list of efficacy endpoints with analysis sets is given in Appendix B.

All calculated exploratory endpoints will be provided by the site.

BMI will be calculated from the height (in meters) and weight (in kilograms) as follows:

$$BMI = kg/m^2$$

The 24-hour laboratory urine assessment parameters, glucose, creatinine and nitrogen (used to calculate protein oxidation), from the metabolic chamber are collected over 6-hour periods but will be summarised as daytime and night-time. The following samples will be grouped:

Daytime = 0-6 hours + 6-12 hours + 12-18 hours

Night-time = 18-24 hours

3.3 Calculation or derivation of safety variable(s)

Safety outcome variables are:

- Incidence of SAEs and DAEs.

- Incidence of independently adjudicated diabetic ketoacidosis (DKA) events, if any.

- Change from baseline in vital signs (pulse, diastolic blood pressure, systolic blood pressure, weight, and BMI). Note: These will also be regarded as efficacy variables, except for pulse.

- Change from baseline in hematology parameters (Hemoglobin and Hematocrit). Note: These will also be regarded as efficacy variables.

- Change from baseline in clinical chemistry parameters namely AST, ALT, ALP, Total Bilirubin, Creatinine, Glucose (fasting and non-fasting), and Potassium. Note: Fasting glucose is also regarded as an efficacy variable.

Safety data will also be collected during the study for:

- Physical examination, where any new or aggravated clinically relevant abnormal medical finding will be reported as an AE only if that are SAEs, DAEs or a suspected DKA event occurred.

- Pregnancy urine testing.

3.3.1 Adverse events

SAEs and DAEs experienced by the subjects will be collected throughout the entire study and will be coded by the AstraZeneca designee using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA).

AE data will be categorized according to their onset date into the following periods:

- AEs occurring pre-treatment: (onset date ≥ Visit 1 and before the first day of administration of IP)
- AEs occurring during treatment: (onset date ≥ the first day of administration of IP in treatment period 1) or (onset date ≥ the first day of administration of IP in treatment period 2)

If an AE start date is fully missing, the start date will be imputed as the date of first dose of study medication in treatment period 1. Guidance for partial start dates for AEs is given in Appendix A. Imputed dates will not appear on the listings. Fully missing and partial AE stop dates will not be imputed. AEs ongoing at the end of the study will be highlighted in listings.

AEs occurring in the pre-treatment period will be listed, but not summarized.

3.3.1.1 Diabetic ketoacidosis events

A DKA Adjudication Committee, blinded to the treatment of the subjects, will independently adjudicate all the suspected DKA events reported by the investigators, as well as those identified based on pre-defined algorithm during the study period. A separate Adjudication Manual, "Guidance for reporting Diabetic Ketoacidosis (DKA) for AstraZeneca Dapagliflozin

Program", will define and describe the procedure for the handling, reporting, and classification of these cases.

3.3.2 Clinical laboratory safety assessments

Blood and urine samples for determination of clinical chemistry, hematology and pregnancy testing will be taken in accordance with the CSP and sent to laboratory for analysis. Additional safety samples may be collected if clinically indicated at the discretion of the Investigator. The parameters outlined in Table 2 in Section 6.2.1 of the CSP will be assessed.

Changes in hematology and clinical chemistry variables between baseline and EOT assessment will be calculated. No conversion from the reported units will take place.

Absolute values will be compared to the relevant reference range and classified as low (below range), normal (within range or on limits) or high (above range). The reference ranges will be collected in the electronic case report form (eCRF). All values falling outside the reference ranges will be flagged.

For the following liver function tests, the multiple of the upper limit of normal (ULN) range will be calculated for each data point: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBL), and alkaline phosphatase (ALP).

Multiple = value / ULN

e.g., if the ALT value is 72 IU/L and the ULN 36 IU/L, then the multiple is 2.

Hy's Law

A potential Hy's Law (PHL) case is defined as any subject with elevations in AST or ALT ≥3 x ULN together with total bilirubin ≥2 x ULN at any point during the study following the start of study treatment, irrespective of an increase in ALP.

A Hy's Law (HL) case is defined as any subject with elevations in AST or ALT ≥3 x upper limit of normal (ULN) together with total bilirubin ≥2 x ULN, where no other reason can be found to explain the combination of increases.

For PHL and HL, the elevation in AST or ALT must precede or occur on the same day as the elevation in total bilirubin, but there is no specified timeframe in which the elevations must occur. Hy's Law cases will be identified programmatically and through medical monitoring.

3.3.2.1 Strip Sign for Selected Laboratory Data

For selected laboratory test values that have been received with an operator sign as a part of the result (>, ≥, <, or ≤), a process to strip the operator sign will be applied and the resulting numeric values will be used for summary data. The raw value with operator will remain as entered in the database and presented as collected in listings.

3.3.3 ECGs

Electrocardiogram (ECG) measurements will be recorded at the Screening visit.

The outcome of the overall evaluation is to be recorded as normal/abnormal in the eCRF, with any abnormalities being recorded as not clinically significant or clinically significant.

3.3.4 Physical examination

Physical examinations will be performed in accordance with Table 4 in the CSP and will include an assessment of the following: general appearance, abdomen, cardiovascular and respiratory systems.

Any new finding(s), or aggravated existing finding(s), judged as clinically significant by the Investigator, will be reported as an AE only if they are SAEs, DAEs or suspected DKA events.

3.3.5 Vital signs

Vital signs (pulse, systolic blood pressure, diastolic blood pressure, height, and weight) will be obtained in accordance with the schedule provided in Table 4 in the CSP.

Changes in vital signs variables between baseline and scheduled or EOT assessments within the corresponding period will be calculated. Abnormal vital signs values will be reported as AEs only if they are SAEs, DAEs or suspected DKA events.

3.3.6 Medical and Surgical History

Medical history/current medical conditions, as well as surgical history, will be coded using the agreed upon version of MedDRA.

3.3.6.1 Diabetes History

Medical history related to diabetes will be collected at Screening (Visit 1). Information on duration of diabetes, type of diabetes, any complications associated with diabetes, type of complication (Retinopathy, Nephropathy, Neuropathy autonomic or peripheral, Angiopathy and other), and baseline metformin dose category (≤500 mg, >500 - ≤1000 mg, >1000 - ≤2000 mg, >2000 mg), will be tabulated.

3.3.6.2 Specific Medical and/or Surgical History

In addition, specific medical and/or surgical histories that have occurred prior to Visit 1, will have information collected on date of diagnosis, date of first treatment and most recent episode/procedure.

4. ANALYSIS METHODS

4.1 General principles

All statistical evaluation, as well as summaries and tabulations, will be done by qualified personnel at IQVIA. Before breaking the treatment codes following clean file declaration (database lock), all decisions on the evaluability of the data for each individual subject will be made and documented, and each subject will be assigned to the appropriate analysis data sets.

Statistical Analysis Software (SAS) version 9.4 or higher will be used to generate all statistical analyses, data summaries and listings.

4.1.1 Statistical notations and presentation

Summary data will be presented in tabular format by treatment group, as well as sequence and/or period where applicable. Categorical data will be summarized by the number and percentage of subjects in each category. Unless otherwise stated, percentages will be calculated out of the population total for the treatment group or sequence. Continuous data will be summarized by descriptive statistics including n (number of subjects with available data), mean, SD, median, minimum and maximum, as well as first and third quartile where warranted. All screening and safety data will be listed, where applicable. For efficacy endpoints, data only pertaining to the primary variable will be listed. Data listings will be sorted by subject number and treatment sequence.

Minimum and maximum values will be reported to the same degree of precision as the raw data up to a maximum of 3 decimal places. Mean, first quartile, median, third quartile, SD and confidence intervals (CIs) will be reported to one further degree of precision. Percentages will be rounded to 1 decimal place.

All hypothesis testing will be performed using 2-sided tests at the 5% significance level. P-values will be rounded to 4 decimal places.

No analysis visit windows will be derived. All by-visit data will be presented by protocol scheduled nominal visits, apart from EOT visits where unscheduled assessments could be used as per Section 3.1.4. Listings will include unscheduled visits.

4.1.2 Statistical analyses

The main focus for the statistical analyses is to compare the effect of dapagliflozin as compared to placebo after 5 weeks of treatment with regards to primary and exploratory endpoints. All variables will be analyzed using a linear mixed effects model. For the primary endpoint, model assumptions will be checked using conventional methods discussed in Section 4.2.4.1.

4.2 Analysis methods

4.2.1 Subject disposition, demography data and subject characteristics

4.2.1.1 Subject disposition

The subject disposition, including counts and percentages of subjects who completed each of the treatment sequences specifying the number of subjects enrolled, re-screened, randomized, who received/did not receive treatment, who completed study, and who terminated prematurely, including reasons for termination, will be summarized for the Enrolled population.

Subject listings of disposition will be generated for all screened subjects.

4.2.1.2 Demographic and baseline characteristics

Demographic data such as age, gender, race and ethnicity will be summarized overall and by treatment sequence for the EAS and Randomized analysis set.

Various baseline characteristics will also be summarized by treatment sequence for the Randomized and EAS populations. These include diabetes disease history including categorization of metformin dose at baseline, weight, height, BMI, diabetes duration and VO_2 max/total body mass.

Specific medical and surgical histories as well as current medical conditions will be summarized by MedDRA Preferred Term (PT) within the System Organ Class (SOC) level of MedDRA for the Safety analysis set.

Subject's age (years) will be presented as collected on the eCRF at Screening.

Duration of diabetes (years) will be calculated using the screening visit date and the date of diabetes diagnosis (see below).

If date of diabetes diagnosis is…	Then duration of diabetes (years) is…
Complete date	(Date of screening – Date of diabetes diagnosis + 1) / 365.25
Partial date (a) Year and month are not missing, but day is missing (b) Year is not missing, but month and date are both missing (b1) Year of diabetes diagnosis is different from the year of screening (b2) Year of diabetes diagnosis is the same as year of screening	 (a) (Number of months between date of diabetes diagnosis and date of screening + 1) / 12 (b1) Difference between year of diabetes diagnosis and year of screening (b2) 2/12 = 0.17 (2 months)

Subject listings of demographic and baseline characteristics, other baseline characteristics of interest, diabetes history, specific medical and surgical history, medical history, and surgical history will be generated for all randomized subjects.

4.2.2 Prior and Concomitant Medications

The number and percentage of subjects receiving each medication (by ATC classification system codes at the 4th level and generic name) will be presented for each treatment group for the populations specified below. Medications will be classified as follows:

- Prior: Medications with a start date < the first day of IP in treatment period 1.
- Concomitant (during treatment):
  - Treatment period 1: Medications that are still ongoing on the first day of IP and also medications with start date ≥ the first day of IP at Visit 2 and < the first day of IP at Visit 5.
  - Treatment period 2: Medications that are still ongoing on the first day of IP at Visit 5 and also medications with start date ≥ the first day of IP at Visit 5 and ≤ the last day of safety follow-up

If a medication has a missing start date then, unless the stop date of the medication indicates otherwise, this medication will be considered as both prior and concomitant to both treatment period 1 and 2. Similarly, if a medication has a partial start date, then unless the partial start date or the stop date indicate otherwise, this medication will be considered as both prior and concomitant.

Separate tables will be presented for all medications received during the concomitant period for allowed and disallowed medications. Disallowed medications will include medications defined as prohibited according to Section 5.7.2 of the CSP. They will be defined following a physician review (prior to database lock) of the unique combinations of ATC code classifications and generic terms captured. Disallowed and allowed medications will be presented based on the Safety analysis set. Percentages will be calculated relative to the number of subjects in the analysis set.

Medications will be classified according to the World Health Organization (WHO) Drug Dictionary.

All medications will also be listed by subject for the Randomized analysis set.

4.2.3 Treatment Compliance

Treatment compliance will be assessed by the tablet count, and the information will be recorded in the appropriate section of the eCRF.

Compliance is defined as:

Compliance (%) = (Total number of tablets taken/total number of tablets that should have been taken) x 100

The total number of tablets taken will be calculated as the total number of tablets dispensed minus total number of tablets returned, based on the subject drug accountability data collected in the eCRF. The total number of tablets that should have been taken is the extent of exposure, as defined below. Percent compliance will be categorized cumulatively by >90% and >120% and summarized by treatment group for the Safety analysis set and Evaluable analysis set. Note, where a dispensed bottle is not returned, percent compliance for the subject is not calculated for the associated treatment period.

Extent of exposure will be defined as the number of days between the start and the end dates of study therapy plus 1 day for each treatment period:

Extent of exposure (days) = (Last dosing date - First dosing date) + 1.

Overall exposure will be calculated as the sum of the extent of exposure days corresponding to the two treatments, regardless of missing dispensed bottles. Compliance and extent of exposure will be presented for each treatment period, treatment total and overall. Treatment compliance and extent of exposure will also be listed by subject for the Safety analysis set.

4.2.4 Efficacy analyses

4.2.4.1 Analysis of the primary efficacy variable

The primary efficacy variable is delta RD (basal vs high insulin) and the aim of the primary analysis is to compare the delta RD (basal vs high insulin) after 5 weeks of treatment with

dapagliflozin with delta RD (basal vs high insulin) after 5 weeks of treatment with placebo. The analysis of the primary endpoint will be performed using the EAS Clamp population.

Summary statistics will be presented by treatment sequence and treatment period, as well as overall for each treatment group. A boxplot of values in each sequence/ period combination will be presented.

Primary model

The expected difference between treatment groups will be estimated using a linear mixed effects model. This model will have treatment group, treatment sequence and period as fixed effects, as well as random intercept for each subject. This model will assume independent conditional residuals with equal variations in each period and treatment group. In the CSP this model is referred to as "mixed Analysis of Variance (ANOVA)", and the induced covariance structure between the two observations belonging to the same subject is equivalent to the covariance structure in the repeated measurements model defined by Equation 2 in Mueller-Cohrs, PhUSE 2006, commonly referred to as a compound symmetry covariance structure. The model stated above will be implemented using Equation 7 in Mueller-Cohrs, PhUSE 2006.
The model will be implemented using SAS® MIXED procedure. The Residual/Restricted Maximum Likelihood (REML) approach will be applied to estimate the model parameters. The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. Missing data will not be imputed.

Model assumption checks

Model assumptions will be checked using conventional methods, i.e. a visual examination of standardised residuals generated from the model with both fixed and random effects (OUTP; box plots and QQ-plots of residuals) to check for outliers. An outlier will be defined as an observation with a standardized residual that is more than three times the interquartile range above the 75th percentile or below the 25th percentile. If outliers are present, then additional sensitivity analyses will be performed with the outliers excluded, in order to assess their impact on the results.

Presentation of results

The least-squares (LS) means for treatment effect in the respective treatment groups, the corresponding standard errors (SE), and the 95% CIs, will be presented. The difference in LS means between the two treatments will also be generated, with corresponding SEs, 95% CI and p-value tabulated. All LS mean estimates will be obtained from the global mixed effects model, and not from stratification by treatment.

Sensitivity analyses

Sensitivity analyses will be performed using a linear regression model with the within-subject difference, defined in Section 3.1.2, for each study endpoint as response and sequence will be included as a term in the linear model. In addition, a non-parametric test of treatment

difference against zero will be performed (Wilcoxon signed-rank test) using all the data and ignoring the sequence membership.

Presentation of results

For the sensitivity analysis utilizing a linear regression model, the estimate of the intercept from the model to get the LS means for the treatment difference, together with the corresponding SE, 95% CI and p-value, will be presented. For the sensitivity analysis utilizing a non-parametric test, the corresponding p-values will be presented. Sensitivity analyses will be presented with primary analysis results for the primary and exploratory endpoints.

Subgroup analyses

There are no planned sub-group analyses.

Baseline imbalance

Due to the small sample size, imbalances in baseline characteristics between the two sequences are possible. However, it is expected that the cross-over nature of the design, in combination with the additional covariates included in the main model (sequence and period) will account for the possible influence of such an imbalance. Thus, no sensitivity analyses connected to baseline characteristics imbalance will be performed.

4.2.4.2 Analysis of exploratory variables

The exploratory endpoints will be analyzed based on the subsets of the EAS as specified in Appendix B. The analysis approach, and the presentation of the results, will be the same as for the primary endpoint, excluding vital signs and lactate. Vital signs parameters will model change from baseline at Visit 3 and 6 (day 15) and Visit 4 and 7 (EOT - day 30) separately. The lactate parameter will model change from time at Visit 4 and 7 for 0 minutes to 15 minutes and 30 minutes separately. The analysis will allow for subjects to be included in the model for one timepoint if the other timepoint has missing data. Checks of outliers and sensitivity analyses will be performed for all exploratory endpoints. Summary statistics and boxplots for vital signs parameters will be produced for absolute values and change from baseline over timepoints as well as by sequence/ period combination. The lactate parameter will output summary statistics for absolute and change from time 0, at particular visits similar to vital signs. Boxplots will not be produced for lactate or chamber blood parameters.

Area under the curve

The chamber blood parameters are scheduled to occur at Visit 4 and Visit 7. Blood samples are drawn before/after breakfast, before/after lunch, before/after dinner and before bedtime. Area under the curve (AUC) will be calculated using the trapezoidal method using the actual sample times collected. The AUC will be defined as the area between the x-axis, where the before breakfast measurement will be equal to time 0, up to the sample collection time for the before bedtime measurement. The AUC will be calculated using the following formula:

$$\sum_{i=1}^{n}(T_{i+1}-T_i)*\frac{C_{(i)}+C_{(i+1)}}{2}$$

Where $C_{(i)}$ and $C_{(i+1)}$ are the sample collections at time i and i+1, $T_{(i)}$ and $T_{(i+1)}$ are time at a given visit. Subject's with missing data for blood samples at Visit 4 or 7 will be excluded from the AUC calculations.

Multiplicity

Due to the number of endpoints, the false discovery rate (FDR), i.e. the expected proportion of hypotheses for which no treatment effect actually exists despite the p-value in the corresponding test being smaller than alpha, might be rather large. In order to illustrate the issue and aid the interpretation of the results, Benjamini-Hochberg (BH) adjusted p-values (DV Mehrotra and JF Heyse 2004) will be presented alongside the unadjusted p-values (sorted from high to low) for all exploratory endpoints in a separate table, the latter obtained from mixed effect models as described earlier. The BH-adjustment of p-values controls the FDR in the sense that, if FDR is set to 0.1, then rejecting the hypotheses with BH-adjusted p-values less that 0.1 will lead to a proportion of false rejections that is, on average, less or equal to 10%. For illustrative purposes, the BH-adjusted p-values that are less than 0.1 will be indicated in the tabulation. Note: The alpha of 0.05 for analyses carried out on the primary and exploratory endpoints are separate to the FDR procedure.

4.2.5 Safety and tolerability analyses

All safety and tolerability variables (including AEs, laboratory parameters and vital signs) will be summarized descriptively by treatment group for the Safety analysis set.

4.2.5.1 Adverse events

AEs (i.e., SAEs, DAEs and potential DKA events) will be summarized for the on-treatment period defined in Section 3.3.1.

An overall summary table will be produced showing the number and percentage of subjects with at least 1 AE in any of the following categories: AEs, AE with outcome of death, SAEs including event with outcome of death, DKA events verified by adjudication committee, DKA events leading to discontinuation of IP, DAEs and AEs leading to withdrawal from study.

AEs, SAEs, DAEs and AEs with outcome of death will be summarized by SOC and PT assigned to the event category using MedDRA. For each PT, the number and percentage of subjects reporting at least one occurrence will be presented i.e., a subject with multiple occurrences of an AE will only be counted once.

SAEs and DAEs (by PT) will be summarized by maximum intensity. If a subject reports multiple occurrence of the same AE, the maximum intensity will be taken as the highest recorded maximum intensity for each PT (the order being mild, moderate, and severe). Any AEs with unknown intensity, causality or seriousness will be regarded as unknown for tabulations.

Independent evaluation of potential DKA events will also be listed.

A listing of AEs including SAEs, with indicators of death due to AE, discontinuations due to AEs, and DKA events will be presented. A separate listing of SAEs with an onset prior to randomization will also be provided.

4.2.5.2 Laboratory data

All continuous laboratory parameters will be summarized by absolute value at each visit, stratifying by treatment sequence, treatment group and overall treatment group, as per the units collected, together with the corresponding changes from baseline. The summary statistics presented will be the minimum, first quartile, median, third quartile, maximum, mean and SD.

Local laboratory reference ranges will be used for the identification of individual clinically important abnormalities and included in subject listings for each laboratory parameter to allow the identification of low, normal, high, and missing values.

Hy's Law

To identify potential Hy's Law cases, a listing of subjects who have ALT or AST ≥3 x ULN and total bilirubin ≥ 2 x ULN at any time during the study (i.e., not necessarily at the same time) will be provided. This listing will include all visits for this subset of subjects.

4.2.5.3 ECG

The Investigator's assessment of the abnormal ECG evaluations collected at Screening visit with description of abnormality will be included in subject listings of ECG, along with detailing whether any abnormalities were clinically significant or not.

4.2.5.4 Vital Signs

All vital signs parameters will be summarized by absolute value at each visit, stratifying by treatment sequence, treatment group and overall treatment group, together with the corresponding changes from baseline. The summary statistics presented will be the minimum, 1st quartile, median, 3rd quartile, maximum, mean and SD.

All recorded vital signs data will be listed.

5. INTERIM ANALYSES

No interim analysis is planned for this study.

6. CHANGES OF ANALYSIS FROM PROTOCOL

The AUC analysis described in Section 4.2.4.2 was not originally planned as per the exploratory analyses Section 8.4.2 of the CSP but was determined to be useful based on the collection of blood parameters over 7 timepoints throughout the day.

7. REFERENCES

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8. APPENDIX

Appendix A

Partial date conventions for AEs

Missing type	Action
If only the **day part** of the AE **onset date** is missing and occurs in the same month and year as 1. the first dose of study medication within a treatment period, 2. the screening period.	1. The date of first dose of study medication in that period will be used as the onset date of the AE. Otherwise, 2. The start date of the screening period will be used as the onset date of the AE. Else, the first day of the month will be used to complete the onset date of the AE.
If the **day and month parts** of the AE **onset date** are missing and occur in the same year as the first dose of study medication within a treatment period.	The date of the first dose of study medication of the earliest treatment period within the year of AE onset will be used as the onset date of the AE.
If the AE **onset date** is completely missing.	The date of the first dose of study medication in treatment period 1 will be used as the onset date of the AE.

Appendix B

Efficacy endpoints

Procedure: Two step hyperinsulinemic euglycemic clamp with glucose tracer		
Group	**Endpoint**	**Analysis set**
Peripheral insulin sensitivity	-RD basal -RD low insulin -RD high insulin -delta RD (basal vs high insulin) [primary endpoint referred to as cGDR in the CSP]	EAS Clamp
Peripheral insulin sensitivity corrected for insulin levels	-delta RD (basal vs high)/steady-state insulin	
Hepatic insulin sensitivity	-EGP basal -EGP low insulin -EGP high insulin -delta EGP (basal vs low insulin) -delta EGP (basal vs high insulin)	
Urinary parameters	-Glucose excretion rate	
Non-oxidative glucose disposal	-NOGD basal -NOGD low insulin -NOGD high insulin -delta NOGD (basal vs high insulin)	

Procedure: Indirect calorimetry		
Group	**Endpoint**	**Analysis set**
Respiratory exchange ratio	-RER basal -RER low insulin -RER high insulin -delta RER (basal vs high insulin)	EAS Indirect Calorimetry
Insulin-stimulated glucose oxidation	-Glucose (CHO) oxidation basal -Glucose (CHO) oxidation low insulin -Glucose (CHO) oxidation high insulin -delta glucose (CHO) oxidation (basal vs high insulin)	
Insulin-suppressed fat oxidation	-Fatty acid (FA) oxidation basal -Fatty acid (FA) oxidation low insulin -Fatty acid (FA) oxidation high insulin -delta Fatty acid (FA) oxidation (basal vs high insulin)	
Procedure: High resolution respirometry		
Group	**Endpoint**	**Analysis set**
Ex vivo mitochondrial function	- state 3 of complex I - state 3 of complex I + II - state 3 of β-oxidation and complex I - state 3 of β-oxidation and complex I+ II - state 4o - state u of β-oxidation	EAS Muscle Biopsy (high resolution respirometry using muscle fibers)

Procedure: MR Spectroscopy		
Group	**Endpoint**	**Analysis set**
PCr recovery measurement	-Half time of PCr recovery	EAS MRS Mitochondrial (PCr recovery rate)
Muscle fat	- IMCL	EAS Muscle Lipid (IMCL)
Acetylcarnitine	-Average acetylcarnitine (AC)/creatine at rest -Maximal acetylcarnitine (AC)/creatine after exercise -Average acetylcarnitine (AC)/creatine after exercise -First acetylcarnitine (AC)/creatine after exercise	EAS MRS Acetylcarnitine
Liver fat	-IHLC	EAS MRS Liver Lipid (IHLC)
Lactate	Lactate at 0, 15 and 30 minutes	EAS MRS Lactate
Procedure: Body composition (DEXA)		
Group	**Endpoint**	**Analysis set**
	-Fat mass -Lean mass -Total mass -Fat mass percentage -Trunk fat mass -Trunk lean mass	EAS DEXA (body composition)

Procedure: Whole body respirometry (Respiration Chamber)		
Group	**Endpoint**	**Analysis set**
Substrate utilization	-24h energy expenditure -Sleeping metabolic rate (SMR) -Diet induced thermogenesis (DIT) -24h RQ -RQ daytime -RQ night-time -Glucose urine loss daytime -Glucose urine loss night-time -Glucose urine loss (24h) -Protein oxidation daytime -Protein oxidation night-time -Protein oxidation (24h) -Urine Creatinine daytime -Urine Creatinine night-time -Urine Creatinine (24h)	EAS Chamber
Blood parameters	-Glucose -Beta-hydroxybutyrate -NEFA -Insulin -Glucagon -FGF21	
Procedure: Fasting and other blood biomarkers		
Group	**Endpoint**	**Analysis set**
Fasting biomarkers	-hsCRP -Uric acid	EAS Biomarkers
Other	-HbA1c -Hb -Erythrocytes Volume Fraction	

Procedure: Vital signs		
Group	**Endpoint**	**Analysis set**
Vital signs	-Body weight -BMI -Systolic blood pressure -Diastolic blood pressure	EAS

To be listed or summarised only:		
Procedure: VO_2 Maximal test		
Group	**Endpoint**	**Analysis set**
VO_2 Maximal test	-VO_2 Max/lean body mass	EAS
Procedure: Two step hyperinsulinemic euglycemic clamp with glucose tracer		
Group	**Endpoint**	**Analysis set**
Blood parameters measured during clamp	-Insulin low -Insulin high	EAS Clamp
Procedure: Blood parameters		
Group	**Endpoint**	**Analysis set**
Fasting biomarkers	-C-peptide	EAS