CLINICAL DEVELOPMENT ULTRASOUND INSONIFICATION

CLINICAL STUDY PROTOCOL GE-HUI-01

AN OPEN-LABEL, PILOT STUDY TO ASSESS THE EFFECTS OF HEPATIC ULTRASOUND INSONIFICATION ON GLUCOMETABOLIC PARAMETERS IN SUBJECTS WITH T2DM

Statistical Analysis Plan

VERSION 2.0
DATE OF PLAN:

30JUN2021

INVESTIGATIONAL DEVICE:

GE LOGIQ™ E10 Imaging Ultrasound System and the C1-6 Probe

NCT # 04502212

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ABBREVIATIONS

ADE	Adverse device effect
AE	Adverse event
AUC	Area under the curve
CGMS	Continuous glucose monitoring system
CRP	C-Reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CSR	Clinical study report
ECG	Electrocardiogram
EGP	Endogenous glucose production
FAS	Full analysis set
FPG	Fasting Plasma Glucose
HBGI	High blood glucose index
HR	Hazard ratio
HOMA-B	Homeostasis Model Assessment of Insulin Secretion
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
ITT	Intent-to-treat
IxRS	Interactive voice/web response system
LBGI	Low blood glucose index
LLN	Lower limit of normal
OGTT	Oral glucose tolerance test
MedDRA	Medical Dictionary for Medical Affairs
PPS	Per protocol set
PD	Pharmacodynamics
PK	Pharmacokinetics
PT	Preferred Term
SAE	Serious adverse events
SD	Standard deviation
SOC	System Organ Class
TEADE	Treatment-emergent adverse device effect
UADE	Unanticipated Adverse Device Effect

1. INTRODUCTION

The statistical analysis plan (SAP) details the planned analysis required to satisfy the Clinical Study Report (CSR) of Clinical Study Protocol GE-HUI-01: An Open-Label, Pilot Study to Assess the Effects of Hepatic Ultrasound Insonification on Glucometabolic Parameters in Subjects with T2DM.

The content of this SAP is based on the protocol Version 4.0 dated 24Sep2020.

Revision Chronology:

V1.0	29MAR2021	Original

2. STUDY OBJECTIVES AND ENDPOINTS

The overarching aim of this exploratory, pilot study is to determine the effect of hepatic ultrasound insonification on insulin sensitivity, glycemic, metabolic and lipid parameters, as well as on various biomarkers in subjects with T2DM.

2.1. Primary Objectives and Endpoints

To evaluate the effect of hepatic ultrasound insonification on changes from baseline in whole-body insulin sensitivity during a two-step hyperinsulinemic, euglycemic clamp (HE Clamp) with stable isotope labeled glucose tracer, assessed by:

- Glucose disposal rate: insulin ratio during steady state (M/I)
- Endogenous glucose production (EGP)
- Rate of glucose disappearance (R_d)

2.2. Secondary Objectives and Endpoints

To evaluate safety and tolerability of hepatic ultrasound insonification in subjects with T2DM, assessed by:

- Incidence and severity of adverse device effects (ADEs) [including, liver injury]
- Incidence and severity of clinically significant laboratory abnormalities
- Change from baseline in vital signs (blood pressure, temperature, respiratory rate, and heart rate)
- Incidence and severity of clinical findings on physical examination
- Change from baseline in 12-lead electrocardiogram (ECG) parameters; the primary ECG endpoint will be QTcF

To evaluate the effect of hepatic ultrasound insonification on changes from baseline in whole-body insulin sensitivity during a two-step hyperinsulinemic, euglycemic clamp (HE Clamp), assessed by:

- Insulin Sensitivity Index (SI)

- Glucose disposal rate during steady state (M)
- Glucose metabolic clearance rate during steady state (MCR)

To evaluate the effect of hepatic ultrasound insonification on change from baseline in glucose tolerance and insulin secretion, assessed by an oral glucose tolerance test (OGTT)

- Area under the curve (AUC), total and incremental time, e.g., $AUC_{0\text{-}180min}$

To evaluate the effect of hepatic ultrasound insonification on glucose metabolism parameters, assessed by:

- Change from baseline in blood glucose (BG) profiles assessed with continuous glucose monitoring system (CGMS)
    - Time spent in defined glucose ranges
    - Average daily glucose
    - BG variability parameters (e.g., coefficient of variation as percentage of mean level [% CV], etc.)
    - Low blood glucose index (LBGI)
    - High blood glucose index (HBGI)
- Change from baseline in Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)
- Change from baseline in Homeostatic Model Assessment of Insulin Secretion (HOMA-B)
- Change from baseline in fasting plasma glucose (FPG)

2.3. Exploratory/Other Objectives and Endpoints

To evaluate the effect of hepatic ultrasound insonification on glucose/metabolism parameters, assessed by:

- Change from baseline in exploratory biomarkers (blood samples will be stored for potential later analysis), e.g., but not limited to:
    - Glucagon
    - Glucagon-like peptide 1 (GLP-1), total
    - Leptin
    - Ghrelin
- Change from baseline in long-term glucose parameters
    - Hemoglobin A1c (HbA1c)
    - Fructosamine
- Change from baseline in lipid metabolism parameters
    - Free fatty acids (FFAs)
    - Triglycerides (TG)
    - Total cholesterol
    - Low-density lipoprotein (LDL-C)
    - High-density lipoprotein (HDL-C)
    - Very low-density lipoprotein (VLDL-C)

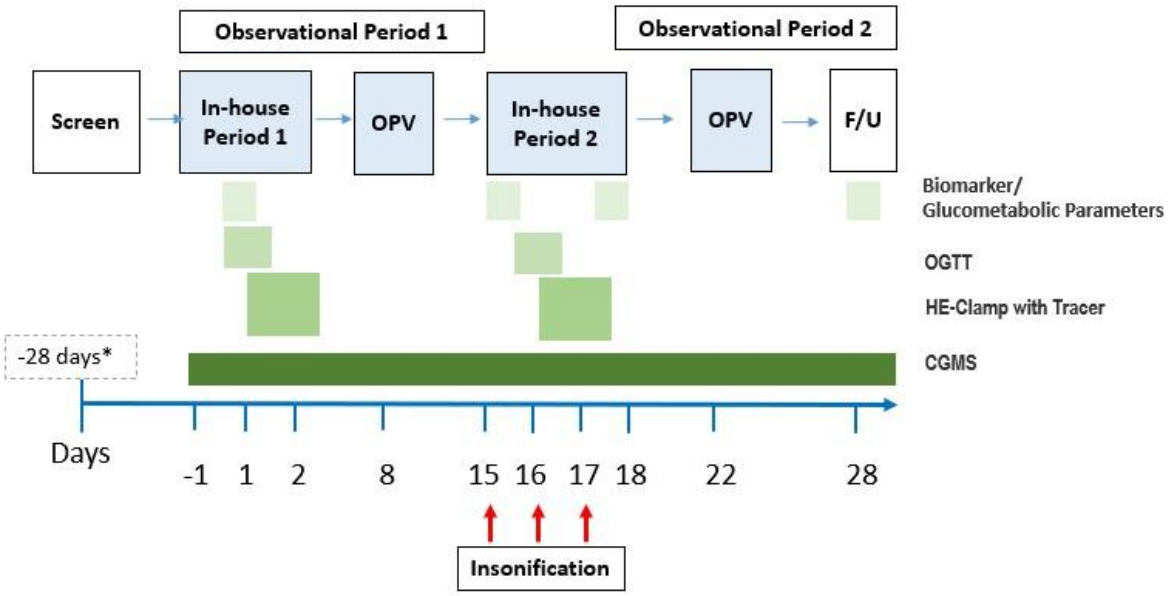
- Change from baseline in inflammatory biomarkers (blood samples will be stored for potential later analysis), e.g., but not limited to:
    - Cytokines (IL-6)
    - Adiponectin
    - C-reactive protein (CRP)

3. STUDY DESIGN

3.1. Experimental Design

This is an open label, exploratory pilot study to assess the effects of hepatic ultrasound insonification on glucometabolic parameters in subjects with T2DM through selective hepatic ultrasound of the porta hepatis region of the liver utilizing pulsed ultrasound.

Figure 1. Study Design Schematic



*Screening Period will be extended for at least 30 days, for subjects with BMI> 35.0 and ≤ 40.0 kg/m2 and/or waist circumference >40 and ≤ 45 inches.

Assessments will be performed via CGMS, OGTT, H-E clamp with stable isotope labeled glucose ([6,6-2H2] glucose), measurements of insulin secretion and resistance (e.g. HOMA-IR), change in glucose parameters, inflammatory biomarkers and potential analysis of exploratory biomarkers.

The study (Fig. 1) will be conducted in 1 cohort, enrolling approximately 15 subjects. Drop-outs may be replaced.

Subjects will undergo a Screening Period of up to 28 days, an approximately two week (Days -1 to 14) Observational Period, followed by three consecutive days of hepatic ultrasound insonification (Days 15, 16 and 17) and a second Observation Period of 11 additional days (Days 18 to 28). The Observational Period is divided in an In-house Period and an Outpatient Visit (OPV). For eligible subjects with a BMI > 35.0 and ≤ 40.0 kg/m2 and/or a waist circumference > 40 and ≤ 45 inches, an ultrasound examination will be added to confirm eligibility, and the screening period will be extended for at least 30 days for these subjects.

3.2. Randomization and Blinding

As this is an exploratory, open-label study, no randomization or blinding will be performed and no-unblinding codes are required. If subjects drop out or are removed from the study, they may be replaced until the anticipated number of subjects have completed the study.

3.3. Sample Size Considerations

No formal sample size calculations were performed as this is an exploratory study. The sample size of 15 subjects was empirically determined and consistent with typical sample sizes used for similar exploratory studies to assess robust data.

3.4. Interim Analysis

There are no planned formal interim analyses for this study.

3.5. Timing of Analyses

The final analysis will be performed once all subjects have completed or discontinued the study and the database has been locked.

4. ANALYSIS POPULATIONS/SETS

Safety Population

The Safety population will consist of all subjects who received at least 1 hepatic ultrasound insonification. This will be the primary analysis population for the evaluation of exposure and safety.

Ultrasound Insonification Effect Population

The ultrasound insonification effect population will consist of all subjects who received at least 1 hepatic ultrasound insonification. It is the same as the safety set and will be the primary analysis population for the primary endpoint.

5. GENERAL DATA HANDLING CONSIDERATIONS

All analyses will be conducted using SAS 9.4 or higher.

All data in the database will be presented in by-subject data listings.

Unless otherwise stated, all listings will be sorted by site ID, subject number, and assessment date (and time, if available).

Unless stated otherwise, continuous data will be summarized by number of subjects (n), mean, median, standard deviation (SD), first quartile (Q1), third quartile (Q3), minimum value, and maximum value.

Unless stated otherwise, categorical data will be summarized n and percentage based on the number of non-missing values. Where applicable, the number of missing values will be presented as a separate category with no percentage Counts of zero will be presented without percentages.

The following precision will be used when presenting data:

- Minimum and Maximum: same number of decimal places as reported in the original data
- Mean, Median, Q1, and Q3: one additional decimal place to that reported for Minimum and Maximum
- SD: two additional decimal places than the Minimum and Maximum
- Percentages: reported to one decimal place
- P-values will be reported to four decimal places. If the value is below 0.0001 it will be noted as < 0.0001; if the value above 0.9999 it will be noted as > 0.9999.

Study data will not be reviewed for purpose of comparison between cohorts/groups. However, if statistical testing is performed, statistical inference will be based on a 5% significance level (i.e. 95% confidence intervals will be produced) and details will be provided in the CSR.

All data up to the time of study completion/withdrawal from the study will be included in the analysis, regardless of duration of treatment.

Numbering for data displays will be based on ICH E3 when applicable.

5.1. Evaluation of Subgroups

There are no formal plans for examining subgroups. If performed, a description will be provided in the CSR.

5.2. Reference Dates

- Screening date is defined as the eCRF provided date on which a subject was screened for trial entry.
- Hepatic ultrasound insonification (HUI) date is defined, for each subject, as the date of first on study HUI.
- Study end date is defined as the subject's date of last study visit or date lost to follow-up.
- Safety data, such as AEs and laboratory assessments will use the subject's first HUI date as a reference date.
- Study day will be defined, in days, relative to each subject's first HUI date.

5.3. Study Day and Duration Variables

For each subject, reference date calculations will generally be defined as the following, assuming non-missing dates:

- date of interest – reference date + 1 when the date of interest >= reference date;
- otherwise, date of interest – reference date.

If either date is missing, reference date calculations will not be performed. Date imputation will be performed as identified in Section 5.5.

Study day based on first HUI date as the reference would either have a negative value if collected before HUI or a positive value if collected on or after the day of first HUI; there will be no study day zero.

Duration of time is dependent on reference dates and will be calculated in a manner similar to that of the reference date calculation, assuming that dates of interest will strictly follow reference dates (e.g. no negative values).

5.4. Baseline, Post-Baseline Changes, and Last Observed Value (LOV)

Unless stated otherwise, baseline and post-baseline change values will be based on the following:

Baseline will be based on the last non-missing value collected prior to the first on study HUI. Post-baseline values will be those collected after initiation of the first HUI.

Change from baseline is defined as: value – baseline value.

Percent change from baseline is defined as: [(value – baseline value)/baseline value] × 100%.

5.5. Imputation of Partial Dates

Adverse Device Effects (ADE) and Concomitant Medications

- If the start date is completely missing, no imputation will be conducted.
- If the start date is missing day and month, do the following:
    - If the start year does not fall in the same year as that of the HUI date or if the record contains information to indicate that the event ended before the HUI date (e.g. the event end date month and year are earlier than the HUI date or the full end date is known and occurs earlier than the HUI date), then set the start month and day to January 1st.
    - Otherwise, set the start date to the HUI date.
- If only the start day is missing, do the following:
    - If the start month and year does not fall in the same month and year as that of the HUI date or if the event contains information to indicate that the event ended before the HUI date, then set the start day to the 1$^{st\ day}$ of the month of the event start date.

    - Otherwise, set the event start date to the HUI date.
- Event end dates will not be imputed.

These imputation rules will be applied similarly to ADE and concomitant medication dates.

5.6. Multiple Assessments and Visit Windows

Nominal visits (e.g. those identified by the study CRF) will be the basis of summarization and statistical analysis; no visit date windowing will be conducted. Unscheduled data may be included in summaries of most extreme and baseline, summaries of specific abnormalities any time post-baseline, and subject data listings.

5.7. Missing Data

AE and concomitant medication date imputations are described in Section 5.5. Otherwise, missing data will not be imputed for any endpoint or analysis.

6. ANALYSIS METHODS

6.1. Subject Disposition

Summaries of analysis population membership and final subject status (completed or withdrawn), including reasons for withdrawal, will be produced. The number of subjects screened will be provided.

Screen failures, analysis populations, and final subject disposition status will be listed.

6.2. Protocol Deviations

Protocol deviations will be identified and classified as important, important due to COVID-19 pandemic, not important, or not important due to COVID-19 pandemic.

Protocol deviations will be summarized by category. A listing of protocol deviations will be provided.

6.3. Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for all subjects. Summary statistics will be generated for continuous variables (e.g., age and weight) and the number and percentage of subjects within each category will be presented for categorical variables (e.g., gender).

The following variables will be summarized:

- Age as a continuous variable

- Gender
- Ethnicity
- Race
- Baseline Height
- Baseline Weight
- Baseline Waist Circumference
- Baseline Body Mass Index (BMI)
- Tobacco/nicotine use
- Marijuana use
- Alcohol use
- Illicit drug use

The following conversions and equations will be used as applicable:

- Height (in cm) = height (in meters) * 100
- Weight (in kg) = weight (in lbs) * 0.4536
- BMI (kg/m^2) = weight(kg)/[height$(m)^2$]
- BSA(m^2) = $\sqrt{}$[(height(cm)*weight(kg))/3600]

6.4. Medical History

Medical history data will be coded based on the Medical Dictionary for Regulatory Affairs (MedDRA) for reporting by system organ class (SOC) and preferred term (PT). A summary of medical history will be prepared and all medical history data will be presented in a data listing.

6.5. Prior and Concomitant Medication

The incidence of medication use will be summarized by WHO Drug Dictionary anatomic therapeutic class (ATC) Level 2 classification (i.e. therapeutic main group) and preferred name. A subject will be counted only once at each level of reporting. Prior medications are those which have been identified to have been discontinued prior to the treatment start date (e.g. taken exclusively during the pre-therapy period). Concomitant medications are those which have been identified to have been taken at any point during the on-therapy or post-therapy periods. Prior and concomitant medication use will be summarized separately and presented by treatment group.

All prior and concomitant medication data will be listed including the verbatim and preferred drug name and ATC Level 2.

6.6. Device Exposure

Listings of all device exposure data will be produced.

6.7. Two-Step Hyperinsulinemic-Euglycemic Clamp Analysis Parameters

The analysis of the two-step H-E Clamp will consist of summarizing whole-body insulin sensitivity and EGP results. Results will be summarized descriptively and described in the appropriate sections below.

Changes in insulin sensitivity will be assessed by the following calculations:

- SI_{clamp}: Insulin Sensitivity Index
- M: Glucose disposal rate during steady state
- MCR: Glucose metabolic clearance rate during steady state
- M/I: Glucose metabolism: insulin ratio during steady state

The steady-state period for insulin sensitivity measurements is defined as the time from 150 to 180 minutes following the initiation of the continuous insulin infusion during each step, and the GIR recorded during the last 30 minutes (steady-state) of each insulin infusion step is used for the determination of insulin sensitivity. Blood samples for the determination of plasma glucose will be drawn at scheduled time points before and during the clamp.

The insulin sensitivity index (SI_{clamp}) will be calculated from the H-E Clamp data using the formula below, with Step 1 indicating the first phase of the clamp, or the period of administration of the first (lower) insulin infusion rate and Step 2 indicating the second phase of the clamp or the period of administration of the second (higher) insulin infusion rate. Mean glucose infusion rates and mean insulin concentrations will be calculated during steady state (the last 30 minutes of the clamp step) for the applicable step(s).

$$SI_{clamp} = \frac{\text{mean(GIR)Step2} - \text{mean(GIR)Step1}}{[\text{mean(I)Step2} - \text{mean(I)Step1}] \times [\text{mean(BG)Steps1\&2}]}$$

Change from baseline (CFB) of SI_{clamp} will then be computed for each subject as

$$CFB(SI_{clamp}) = SI_{clamp2} - SI_{clamp1},$$

where clamp 2 is the second clamp and clamp 1 is the first clamp (completed on Day 2; prior to first insonification). Change from baseline of SI_{clamp}, together with SI_{clamp} for each clamp individually, will be included in the efficacy analysis dataset.

Glucose disposal rate (M) during steady state

Glucose disposal rate (M) is obtained as the glucose infusion rate per min corrected for body weight and/or fat free mass. Glucose disposal rate will be calculated separately for Step 1 and Step 2 and for each clamp as the average of the glucose infusion rate per min corrected for body weight and/or fat free mass during the last 30 minutes of the clamp step. The values calculated for Step 1 and Step 2 during the clamp procedure on Day 2 will serve as baseline values and will be compared to the respective Step 1 and Step 2 values on Day 17.

Glucose metabolic clearance rate (MCR) during steady state

Glucose metabolic clearance rate (MCR) is calculated using the formula below for each clamp. Rates will be compared. Glucose MCR will be calculated separately for Step 1 and Step 2, with Step 1 indicating the first phase of the clamp, or the period of administration of the first (lower) insulin infusion rate and Step 2 indicating the second phase of the clamp or the period of administration of the second (higher) insulin infusion rate. The values calculated for Step 1 and Step 2 during the clamp procedure on Day 2 will serve as baseline values and will be compared to the respective Step 1 and Step 2 values on Day 17.

$$MCR = 100 \times \frac{mean(M)}{mean(BG)}$$

Where M is the glucose infusion rate per min corrected for body weight and/or fat free mass and BG is blood glucose.

Glucose metabolism: insulin ratio (M/I) during steady state

Insulin ratio (M/I) will be calculated for each clamp and ratios will be compared. Ratios will be calculated separately for Step 1 and Step 2. This is the preferred measure and primary endpoint. Glucose metabolism: insulin ratio (M/I) is calculated as the glucose disposal rate (M) divided by the insulin concentration (I). . The values calculated for Step 1 and Step 2 during the clamp procedure on Day 2 will serve as baseline values and will be compared to the respective Step 1 and Step 2 values on Day 17.

6.8. Primary Endpoints and Analyses

6.8.1. Glucose disposal rate: insulin ratio during steady state (M/I)

M/I calculations are described above. The ratio during steady state will be calculated for each step of each clamp and summarized using descriptive statistics in the Ultrasound Insonification Effect Population.

6.8.2. Endogenous glucose production (EGP)

Fasting EGP

Prior to the two-step H-E Clamp, fasting EGP will be assessed utilizing a low primed-continuous infusion of a stable isotope-labeled glucose. At each blood collection time point time point, plasma glucose enrichments will be measured to determine steady state and to determine fasting EGP using steady-state equations (Steele 1959).

EGP will be summarized using descriptive statistics in the Ultrasound Insonification Effect Population.

Insulin-mediated EGP Suppression

For each clamp, the degree of EGP suppression from the fasting EGP value will be determined during the last 30 minutes point during each step of the two-step H-E Clamp using the following equation:

$$\% \text{ EGP suppression} = 1 - (\text{EGP clamp}/\text{EGP fasting})$$

Partial suppression of EGP during Step 1 is assessed to determine hepatic insulin sensitivity and EGP suppression during Step 2 will be measured to confirm EGP was (near) fully suppressed to allow the determination of extrahepatic insulin sensitivity.

%EGP suppression will be summarized using descriptive statistics in the Ultrasound Insonification Effect Population.

6.8.3. Rate of glucose disappearance (Rd)

Under steady state conditions, the Rate of Glucose Disappearance (Rd) is equal to the Rate of Glucose Appearance (Ra) and under these conditions Ra is calculated from the dilution of plasma [6,6-2H2]glucose during constant infusion of [6,6-2H2]glucose. Rd will be calculated during steady state in the fasting period and during Step 1 and Step 2 of the clamp.

Rd will be summarized using descriptive statistics in the Ultrasound Insonification Effect Population.

6.9. Secondary Endpoints and Analyses

Adverse device effects

The incidence and severity of adverse device effects (ADEs) will be summarized. ADEs/AEs will be considered treatment emergent if their onset occurs after initiation of the first insonification.

Any missing severity assessments will be assumed to be Grade 3, missing relationship assessments will be assumed to be related, and missing seriousness assessments will be assumed as serious in the relevant summaries.

An overview of treatment-emergent ADEs (TEADE) will be produced, including counts and percentages of subjects with any incidences of:

- TEADE
- TEADE related to study procedure
- Serious TEADE
- TEADEs leading to study discontinuation

ADE summaries will include the overall incidence (by system organ class and preferred term), events by maximum intensity, event by relationship to study procedure, events leading to discontinuation of study, and serious ADEs. ADEs will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v5.0).

Adverse events will be coded based on MedDRA. TEADEs will be summarized by system organ class (SOC) and preferred term (PT) in descending order of overall incidence. For these summaries, subjects will be counted once within a SOC/PT based on their TEAE having maximum severity or relationship, as applicable.

Summaries of adverse events by SOC and PT will include the following types:

- TEADEs;
- TEADEs related to study procedure
- CTCAE Grade 3 or higher TEADEs
- CTCAE Grade 3 or higher TEADEs related to study procedure
- Serious TEADEs
- TEADEs leading to study discontinuation
- UADEs

A summary of TEADEs by SOC, PT, and maximum severity will be prepared. Events with missing severity will be assumed to be Grade 3. A summary of TEADEs by SOC, PT, and maximum relationship will also be prepared. Events with missing relationship will be assumed to be Related.

A comprehensive listing of all ADEs will be provided in a by-subject data listing. In addition, the following listings will be provided:

- TEADEs related to study procedure
- Serious TEADEs
- TEADEs leading to study discontinuation

Clinically significant laboratory abnormalities
Laboratory abnormalities will be graded and evaluated for clinical significance.

The number and percent of subjects of subjects with a clinically significant laboratory abnormality will be summarized by laboratory parameter and study visit. In addition, a summary of laboratory toxicity grades will be prepared by laboratory parameter and study visit.

The denominator for percentages will be the number of subjects with data for the given parameter and visit.

Clinical chemistry and hematology parameters will be reported based on the International System of Units (SI). Observed values and changes from baseline for laboratory evaluations will be summarized at each visit post initiation of HUI. Baseline is defined as the last non-missing value collected prior to first hepatic ultrasound insonification.

A listing of laboratory abnormalities will be provided as will a listing of all laboratory parameters.

Vital signs

Absolute values and changes from baseline will be summarized for vital signs parameters at specified timepoints in the protocol Schedule of Events.

Vital signs include: systolic and diastolic blood pressure (mmHg), temperature (C), respiratory rate (bpm), and heart rate (bpm). Observed values and changes from baseline for vital signs will be summarized at each visit and time point.

All vital signs data will be listed.

Physical examination

A summary of physical examination (PE) findings will be presented by body system as recorded on the eCRF. The number of subjects and percent will be summarized based on abnormal examination results (abnormal without clinical significance and abnormal with clinical significance) by visit. The number and percent of subjects with any abnormal finding will also be provided. The denominator will be the number of subjects with a physical examination performed at the visit.

Physical examination findings will be listed by subject.

Electrocardiogram (ECG)

Absolute values and changes from baseline will be summarized for 12-lead ECG parameters at specified timepoints. The primary ECG endpoint will be QTcF.

ECG parameters include: QT interval (ms), PR interval (ms), QRS interval (ms), RR interval (ms), QTcF (ms). Observed values and changes from baseline for ECG data will be summarized at each visit and time point.

In addition to the continuous summary, the overall interpretation of ECG results will be summarized as within normal limits, abnormal without clinical significance, and abnormal with clinical significance by visit and time point.

All ECG data will be listed.

Whole-body insulin sensitivity

The effect of hepatic ultrasound insonification on changes from baseline in whole-body insulin sensitivity during a two-step HE Clamp will be assessed by:

- Insulin Sensitivity Index (SI)
- Glucose disposal rate during steady state (M)
- Glucose metabolic clearance rate during steady state (MCR)

The formulas for SI, M, and MCR are described in section 6.8. Each parameter will be summarized using descriptive statistics in the Ultrasound Insonification Effect Population.

Glucose tolerance and insulin secretion

To evaluate the effect of hepatic ultrasound insonification on change from baseline in glucose tolerance and insulin secretion, assessed by an oral glucose tolerance test (OGTT), area under the curve (AUC) total and incremental time (e.g., $AUC_{0\text{-}180min}$) will be calculated.

AUC is calculated according to the linear trapezoidal rule:

$$AUC(t_i - t_{i+1}) = \frac{C(C_i) + C(C_{i+1})}{2} \times (t_{i+1} - t_i)$$

where C is glucose concentration (mg/dL) with measurements at times $t_1,..t_n$ such that

$$AUC_{total} = \sum_{i}^{n-1} AUC(C_i - C_{i+1})$$

$AUC_{0\text{-}180min}$ is similarly derived but uses data only through 180 minutes.

AUC results will be summarized using descriptive statistics in the Ultrasound Insonification Effect Population.

Glucose metabolism parameters

The effect of hepatic ultrasound insonification on glucose metabolism parameters will be evaluated by the following endpoints in the Ultrasound Insonification Effect Population:

- Blood glucose (BG) profiles assessed with continuous glucose monitoring system (CGMS)
    - Time spent in defined glucose ranges
    - Average daily glucose
    - BG variability parameters (e.g., coefficient of variation as percentage of mean level [% CV], etc.)
    - Low blood glucose index (LBGI)
    - High blood glucose index (HBGI)
- Homeostasis Model Assessment of Insulin Resistance (HOMA2-IR)
- Homeostatic Model Assessment of Insulin Secretion (HOMA2-B)
- Fasting plasma glucose (FPG)

The baseline period for CGMS parameters will be calculated using data starting from 14:00h on Day -1 to 22:00h on Day 1, omitting data collected between a subjects OGTT glucose consumption start time + 180 minutes.

The primary insonification analysis period for CGMS parameters will be from 14:00h on Day 15 to 22:00h on Day 16, omitting data collected between a subjects OGTT glucose consumption start time + 180 minutes.

The CGMS parameters will be summarized in a table using descriptive statistics for the primary insonification analysis period and change from baseline. The CGMS parameters for the baseline period, primary insonification analysis period and Days 19 and 20 will be listed.

The percent of time spent in defined glucose ranges will be calculated separately for each of the analysis periods along with the change from the baseline period. The following glucose ranges will be defined and presented separately:

- > 250 mg/dL (>13.9 mmol/L)
- > 180 mg/dL (>10.0 mmol/L)
- 70-180 mg/dL (3.9-10.0 mmol/L)
- < 70 mg/dL (<3.9 mmol/L)
- < 54 mg/dL (<3.0 mmol/L)

Average daily glucose will be calculated based on the applicable glucose measurements obtained during the specified day/period.

To calculate the LBGI and HBGI, the original BG data for each day/period is transformed such that it is centered by zero and bound between $-\sqrt{10}$ and $\sqrt{10}$.

$$f(BG, \alpha, \beta) = [(\ln(BG))^{1.084} - 5.381] * 1.509$$

The transformed values are then inputted into the BG risk function:

$$r(BG) = 10 * f(BG, \alpha, \beta)^2$$

The risk of each observation is further transformed into two series, risk of low BG series, rl(BG), and risk of high BG series, rh(BG):

$$rl(BG) = r(BG) \; if \; f(BG) < 0 \; and \; 0 \; otherwise$$

$$rh(BG) = r(BG) \; if \; f(BG) > 0 \; and \; 0 \; otherwise$$

These series are then averaged to produce the LBGI and the HBGI as follows:

$$LBGI = \frac{1}{n}\sum_{i=1}^{n} rl(x_i)$$

$$HBGI = \frac{1}{n}\sum_{i=1}^{n} rh(x_i)$$

Absolute values and changes from baseline will be summarized using descriptive statistics for BG, HOMA2-IR, HOMA2-B, and FPG. Baseline is defined as the last non-missing value collected prior to a subject's first HUI. The HOMA2 results will be produced by the HOMA2 calculator with fasting plasma glucose and insulin concentration obtained from safety laboratory

assessments as the inputs. Summaries will be provided by study visit/time point as applicable in the protocol Schedule of Events.

For BG, coefficient of variation (CV) calculated as 100 x [standard deviation (SD) divided by mean] will be included with the other summary statistics.

6.10. Exploratory/Other Endpoints and Analyses

Glucose/metabolism parameters

The effect of hepatic ultrasound insonification on glucose metabolism parameters will be evaluated by evaluation of absolute values and changes from baseline. Baseline is defined as the last non-missing value prior to first hepatic ultrasound insonification. Summaries will be provided for the following parameters by study visit/time point:

- Exploratory biomarkers:
  - Glucagon
  - Glucagon-like peptide 1 (GLP-1), total
  - Leptin
  - Ghrelin
- Long-term glucose parameters:
  - Hemoglobin A1c (HbA1c)
  - Fructosamine
- Lipid metabolism parameters:
  - Free fatty acids (FFAs)
  - Triglycerides (TG)
  - Total cholesterol
  - Low-density lipoprotein (LDL-C)
  - High-density lipoprotein (HDL-C)
  - Very low-density lipoprotein (VLDL-C)

Summaries will be based on the Ultrasound Insonification Effect Population.

Inflammatory biomarkers

The effect of hepatic ultrasound insonification on inflammatory biomarkers will be evaluated by evaluation of absolute values and changes from baseline. Baseline is defined as last non-missing value collected prior to first hepatic ultrasound insonification. Summaries will be provided for the following parameters by study visit/time point:

- Cytokines (IL-6)
- Adiponectin
- C-reactive protein (CRP)

Summaries will be based on the Ultrasound Insonification Effect Population.

7. CHANGES TO THE PLANNED ANALYSIS

Analyses and data presentations described in the protocol have been expanded upon, but no other changes were made to analyses specified in the protocol. Any changes to the planned analyses as detailed in this SAP will be described in the CSR.