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

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NCT # 04502212

1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

A separate contact information list will be provided.

1.2 Document History

Version	Revision Date	Revision Description
1.0	**13-Mar-2020**	**Original Document**
2.0 (Amendment 1)	**26-May-2020**	**1. Revisions to the Protocol for Submitting an Investigational Device Exemption (IDE) Application** Language in regard to nonsignificant risk assessment has been taken out. *Section 1.3 Approval, section 4.4 Summary of Known and Potential Risks and Benefits, section 10.3.6 Summary of Pre-Clinical /Clinical Studies, section 14.0 Ethical Aspects of the Study, section 14.2 Regulatory Authorities* **2. Fitness Tracking has been canceled** The recording of physical activity and sleep via fitness tracking device will not be performed in this study *Section 2.0 Study Summary, section 6.2 Study Description, section 9.1.18 Physical Activity and Sleep Recording, section 16.0 SOE in the Appendix* **3. Clarification of Inconsistencies** Adjustment of protocol language in order to clarify inconsistency between protocol language and procedures stated in the Schedule of Events (SOE) around assessments on Days 8 and 22. *Section 2.0 Study Summary, section 6.2 Study Description* **4. Change to Inflammatory Biomarkers** Blood samples for the assessment of inflammatory biomarkers may be stored for potential later analysis. Additionally, IL-1β will

		not be analyzed. *Section 2.0 Study Summary, section 5.3 Exploratory/Other Objectives and Endpoints, section 9.1.8 Procedures for Clinical Laboratory Samples, section 9.1.24 Assessments of Ultrasound Insonification Effects* **5. Clarification/Explanation of Terminology** Additional language has been added to specify the term plasma glucose. *Section 9.1.15 Self -Monitoring of Blood Glucose* **6. Specification of Monitoring process** Additional language has been added to specify the monitoring procedures for the study. *Section 13.1 Monitoring* *7.* **Correction of Typographical Errors and adjustment of wording for better clarity** Correction throughout the entire document.
3.0 (Amendment 2)	**25-June-2020**	**Revisions to the protocol based on feedback from the US FDA** **1. Change to the Inclusion Criterion #3** Only subjects on stable treatment with diet and exercise or stable treatment with metformin monotherapy are included, as judged by the qualified Principal Investigator. Subjects on other oral anti diabetic drugs (OADs) will not be included in the study. *Section 2.0 Study Summary, section 7.1 Inclusion Criteria, section 7.3 Prohibited Medication* **2. Adding of Exclusion Criteria #2:** A subject who is already indicated for medication escalation of their current diabetic therapy, or, who based upon study entry criteria, would be indicated for medication escalation during the course of the study (as assessed by the Principal Investigator).

		Section 2.0 Study Summary, section 7.2 Exclusion Criteria **3. Removal of Wash-out Period** As subjects taking OADs in addition to metformin will not be included, the wash-out period is not needed and has been removed. *Section 2.0 Study Summary, section 6.1 Study Design, section 6.2 Study Description, section 6.3 Rationale for Study Design and Endpoints, section 9.1.13 Wash-Out Period, section 9.1.14 Self-monitoring of Blood Glucose (SMBG), section 16.0 Appendix* **4. Change of FPG Threshold** FPG level for which subjects are instructed to contact study staff will be lowered from 270 mg/dL to 220 mg/dL. *Section 2.0 Study Summary, section 6.2 Study Description, section 9.1.14 Self-monitoring of blood glucose (SMBG)* **5. Shortening of Screening Period** The screening period will be shortened to 28 days, as the wash-out period is removed. *Section 2.0 Study Summary, section 6.1 Study Design, section 6.2 Study Description, section 16.0 Appendix* **6. Revision of Duration of Participation** The overall duration of participation in this study will be shorter for each subject due to the withdrawal of the wash-out period and consecutive shortening of screening period. *Section 2.0 Study Summary* **7. Revisions to reflect the abbreviated requirements per IDE Regulations** Language in regard to nonsignificant risk assessment has been added. *Section 1.3 Approval, section 4.4 Summary of Known and Potential Risks and Benefits, section 14.0 Ethical Aspects of the Study*

		Other Revisions **8. Adjustment of Language around Telephone Visits/Calls** Wording added to check for subject's BG in order to provide consistent language for visits throughout the protocol. *Section 2.0 Study Summary, section 6.2 Study Description* **9. Adjustment of Language for Exclusion Criterion** History or active hepatic disease added to the exclusion criterion #14 in the main protocol in order to have consistent language in synopsis and main protocol. *Section 7.2 Exclusion Criteria* **10. Removal of Specific Laboratory Parameters to be collected for Exploratory/Other Endpoints** For the inflammatory biomarkers, blood samples will not be analyzed for tumor necrosis factor alpha (TNF-α) and norepinephrine. For long-term glucose parameters, blood samples will not be analyzed for 1,5-Anhydroglucitol. *Section 2.0 Study Summary, section 3.0 List of Abbreviations, section 4.3 Summary of Pre-Clinical /Clinical Studies, section 5.3 Exploratory/Other Objectives and Endpoints, section 9.1.8 Procedures for Clinical Laboratory Samples, section 9.1.23 Assessments of Ultrasound Insonification Effects, section 16.0 Appendix*
4.0 (Amendment 3)	**24-Sep-2020**	**Revisions:** **1. Changes to the Inclusion Criterion # 5 and Inclusion Criterion #6** Criterion for BMI will be widened to BMI ≤ 40.0 kg/m^2. Criterion for inclusion of waist circumference will be widened to ≤ 45 inches (≤ 114 cm) to account for sedentary lifestyle of subjects due to COVID-19. Subjects with a

		BMI > 35.0 kg/m^2 (old criterion) and ≤ 40.0 kg/m^2 (new criterion) and/or a waist circumference between > 40 inches (old criterion) and ≤ 45 inches (new criterion) will need to undergo an ultrasound examination for eligibility (including visibility of the porta hepatis and a measured distance from the skin surface to the porta hepatis target not to exceed 10 cm), followed by a 30-day wait period starting after the ultrasound examination has been performed, in order to comply with exclusion criterion # 35. Eligibility criteria will be re-assessed after the wait period. Therefore, the screening period will be extended for these subjects. *Section 2.0 Study Summary, section 6.1 Study Design, section 6.2 Study Description, section 7.1 Inclusion Criteria, section 9.1.2 Screening, section 9.1.26 Hepatic Ultrasound at Screening, section 16.0 Appendix* **2. Change of Exclusion Criterion #3** Subjects with moderate peripheral neuropathy will be excluded and only subjects with mild peripheral neuropathy may be included in the study. The Toronto Neuropathy Scoring System will be used for the assessment of the neuropathy and is referenced in the Appendix. *Section 2.0 Study Summary, section 7.2 Exclusion Criteria, section 9.1.4 Physical Examination, section 16.0 Appendix* **3. Clarification to Ultrasound Examinations** Ultrasound examination during the first ultrasound insonification will be performed to ensure that the target area can be visualized or stimulated and a measured distance from the skin surface to the porta hepatis target does not exceed 10 cm. *Section 6.2 Study Description, section 9.1.24 Hepatic Ultrasound Insonification* **4. Incorporation of Protocol Clarification Letter # 1, dated 25 Aug 2020:**

		a) Insonification Approximate Start Time Insonification may start at approximately 07:00 h, but may be staggered and performed between 07:00h and 08:30 h to allow an extra 30 min to allow for COVID-19 testing procedures prior to insonification. *Section 2.0 Study Summary, section 6.2 Study Description, section 8.4 Regimen of Hepatic Ultrasound Insonification, section 9.1.24 Hepatic Ultrasound Insonification* **b) Exclusion Criteria #1 – History of Insulin** Clarification of insulin use prior to the study: Subjects who have participated in studies with insulin or given insulin during hospitalization (e.g. to manage diabetes when oral therapy is not allowed) outside of a 3-month window should not be excluded from participation as this is not considered ongoing treatment. *Section 2.0 Study Summary, section 7.3 Prohibited Medication* **c) Rescheduling Visits – Insonification Visits (Day 15, 16, 17)** Subjects will be given up to 5 days to reschedule check-in on Day 15, in case of a weekend/holiday or subject's personal issue. However, once checked-in for Day 15, there would not be a window to reschedule remaining insonification procedures (Day 16 & 17). *Section 9.1.12 Check-in Procedure*

1.3 Approval

Representatives of Sponsor and Principal Investigator will sign the agreement on the protocol.

Investigator Approval Page

Protocol Number:	GE-HUI-01
Protocol Title:	An Open-Label, Pilot Study to Assess the Effects of Hepatic Ultrasound Insonification on Glucometabolic Parameters in Subjects with T2DM
Protocol Version:	4.0
Date:	24 Sep 2020

The Principal Investigator agrees to conduct this Study as outlined in this protocol in reference to national/local regulations and in accordance with current Good Clinical Practice (GCP) guidelines described in the International Committee for Harmonization (ICH) Guidance document E6 (R2), the abbreviated FDA regulations for clinical trials, 21 CFR 812.2(b), the Health Insurance Portability and Accountability Act (HIPAA), the most current version of the Declaration of Helsinki, and the terms of the Clinical Study Agreement entered on December 19, 2019 as may be amended from time to time by and between ProSciento, Inc., and General Electric Company, acting through GE Research. Any modification to the protocol must be agreed upon by both the Principal Investigator and Sponsor and documented in writing. By written agreement to this protocol, the Principal Investigator agrees to allow direct access to all documentation, including source data, to authorized individuals representing the Sponsor (including monitoring staff and auditors) only for the purpose of monitoring and auditing the Study, to Institutional Review Boards (IRB) and/or to regulatory authorities.

Principal Investigator:

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Bridgette Franey, MD	BFraney	25Sep2020
Name (printed)	**Signature**	**Date**

Sponsor Protocol Approval Page

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Approved for the Sponsor by:

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2.0 STUDY SUMMARY

Name of Investigational Product	Hepatic Ultrasound Insonification using General Electric LOGIQ E10 with C1-6 Probe
Protocol Number	GE-HUI-01
Protocol Title	An Open-Label, Pilot Study to Assess the Effects of Hepatic Ultrasound Insonification on Glucometabolic Parameters in Subjects with T2DM
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 (H-E 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)
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 (H-E 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)
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)
Phase of Development	1
Number of Study Sites	1
Study Population	Subjects with T2DM

Number of Subjects	Approximately 15 subjects will participate in this study. Drop-outs may be replaced in order to achieve 15 completed subjects.
Summary of Study 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. Assessments will be performed via CGMs, OGTT, HE-clamp with stable isotope labeled glucose ([6,6-$^2H_2$] 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 will be conducted in 1 cohort, enrolling approximately 15 subjects. Subjects will undergo a Screening Period of up to 28 days, an approximately two week (Days -1 to 14) Observational Period, follow by three consecutive days of ultrasound Insonification (Days 15, 16 and 17) and a second Observation Period of 11 additional days (Days 18 to 28). Screening: A Screening Visit will be performed up to 28 days prior to the first in-house period to identify eligible subjects for the study. For eligible subjects with a BMI > 35.0 and ≤ 40.0 kg/m^2 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. In-house Period 1: Day -1: Subjects will check in to the clinic in the morning on Day -1 for a 3-day in-house period. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Subjects get instructions on maintaining their regular diet and physical activity. Subjects will receive standardized meals throughout their in-house stay. Food intake will be recorded during the entire in-house period by study staff. Subjects will be connected to a CGM (e.g., Dexcom G6) for the entire study period. Subjects will be blinded to the CGMS data. Continuous glucose monitoring data from Day -1 to Day 14 will be used for the baseline assessment. Subjects will be provided with a glucometer. They will be instructed on how to use the glucometer and to monitor their fasting plasma glucose levels daily during the outpatient periods. They will be instructed to contact the study staff, if FPG > 220 mg/dL, they experience symptoms of hypoglycemia, or measures an FPG < 70 mg/dL. Subjects will be frequently contacted by qualified study site staff to review their fasting plasma glucose values. Blood samples will be taken for safety assessments. Subjects will be instructed to fast overnight.

	Day 1: Following ≥ 10 hour overnight fast, in the morning on Day 1, fasting blood samples will be taken for the assessment of various metabolic parameters as well as for banking for future exploratory measurements. Thereafter, an OGTT will be performed over 180 min as a baseline assessment. Standardized meals will be provided following the OGTT and in preparation of the clamp, that will be performed on the next day. Approximately around/after 22:00 h, subjects will receive a low-dose overnight intravenous (IV) infusion of insulin to titrate the BG to a target level of 100 mg/dl (± 10%) in preparation for the H-E clamp. Approximately at midnight, a primed-continuous IV infusion of stable isotope labeled glucose ([6,6-$^{2}H_{2}$]glucose) will also be initiated for the measurement of EGP. Day 2: After completing the fasting EGP assessments, subjects will undergo a 6-hour H-E clamp with stable isotope labeled glucose. Following their glucose clamp, subjects will be monitored post clamp to ensure adequate glycemic stabilization. They will be provided with a standardized meal and may then be released home. Telephone Visits: Subjects will be contacted every other day until they come in for the next in-house period, to remind the subjects of their diet and exercise regimes and to check subjects BG. Outpatient Visit(s): Subjects will check-in on Day 8 for their outpatient visit. A sensor change of the CGMS device will be performed. Blood samples will be taken for safety assessments. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Subjects will be re-instructed to maintain their usual diet and physical activity regimes. In-house Period 2: Day 15: Subjects will check in to the clinic in fasting conditions on Day 15 for a 4-day in-house period. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Blood samples will be taken in the fasting state prior to any assessment for the analysis of FPG, safety parameters, various metabolic parameters, as well as for banking for future exploratory measurements. They will get re-instructed to maintain their regular diet and physical activity. Subjects will receive standardized meals throughout their in-house stay. Food intake will be recorded during the entire in-house period by study staff. Subjects will undergo the first of three ultrasound insonifications (following ≥ 10 hour overnight fast). Insonifications will be performed on three consecutive days (Days 15, 16 and 17). Subjects will be insonified by the stimulus pulse for 15 minutes on each day for a total of 45 minutes per study. Breath holds and image targeting will extend the total duration that the probe is on the subject (without the stimulus). Insonification will be performed, starting at approximately between 07:00 h and 08:30 h.

	Day 16: Following ≥ 10 hour overnight fast, in the morning of Day 16, subjects will undergo the second of three ultrasound insonifications. Thereafter, an OGTT will be performed over 180 min. Standardized meals will be provided following the OGTT and in preparation of the clamp, that will be performed on the next day. Approximately around/after 22:00 h, subjects will receive a low-dose IV infusion of insulin to titrate the BG to a target level of 100 mg/dl (± 10%) in preparation for the H-E clamp. Approximately at midnight, a primed-continuous IV infusion of stable isotope labeled glucose will also be initiated for the measurement of EGP. Day 17: Following ≥ 10 hour overnight fast, in the morning of Day 17, subjects will undergo the last of three ultrasound insonifications. All insonifications will be performed around the same time on each day. Subjects will be monitored for any evidence of hypoglycemia, liver injury, or any other device related adverse effect. Thereafter, subjects will undergo fasting EGP measurements and a 6-hour H-E clamp. During the clamp procedure, IV infusion with stable isotope labeled glucose will be continued to assess EGP suppression at Step 1 and Step 2 of the clamp. Following the glucose clamp, subjects will be monitored post clamp to ensure adequate glycemic stabilization. Subjects will receive a standardized meal at the end of the procedure. Day 18: Blood samples will be taken in the fasting state for safety assessments, metabolic parameters as well as for banking for exploratory biomarkers. A sensor change of the CGMS device will be performed. Subjects may be released home or may be asked to stay in-house for an extended time, if deemed appropriate and needed by the Principal Investigator to monitor for the risk of hypoglycemic episodes. Telephone Visits: Subjects will be contacted every other day until the Follow-up Visit, to remind the subjects of their diet /exercise and to check subjects BG. Outpatient Visit(s): Subjects will check-in on Day 22 for their outpatient visit. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Blood samples will be taken for safety assessments. Subjects will be re-instructed on maintaining their regular diet and physical activity. Follow-up Visit: A Follow-up Visit will be performed on day 28 for final safety procedures, blood sampling in fasted state for safety, metabolic parameters as well as for banking for future exploratory biomarkers will be performed. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. CGM data will be uploaded to site computer via USB transfer from CGM receiver.

	Subjects will be instructed to transition back to their regular diabetes medication regimen. Sampling and Assessment: Safety assessments will occur throughout the duration of the study, including monitoring of adverse device effects (ADEs), clinical laboratory tests (chemistry, hematology, and urinalysis), vital signs measurements (blood pressure, heart rate, respiration rate, and aural temperature), 12-lead electrocardiograms (ECGs), and physical examinations. Unscheduled outpatient visits: Additional, unscheduled outpatient visits may occur, in the event the CGMS might get dislodged and the sensor would need to be re-inserted by clinic staff.
Treatment/ Intervention	Selective ultrasound stimulation of the porta hepatis region.
Route of Administration	Ultrasound probe will be placed against the abdomen in order to administer insonification.
Duration of Participation	The duration of participation in this study, including Screening, Treatment, Observation and Follow-up will approximately be up to 8 weeks for each subject. For subjects with a BMI > 35.0 and ≤ 40.0 kg/m^2 and/or a waist circumference > 40 and ≤ 45 inches, the duration of participation in this study will approximately be 12-13 weeks.
Inclusion Criteria	Subjects who meet all the following criteria at Screening to be eligible to participate in the study: 1. Male or female subjects with type 2 diabetes ≥ 12 months. 2. Age ≥ 21 and ≤ 75 years. 3. Stable treatment with diet and exercise or stable treatment with metformin monotherapy. Stable treatment is defined as no change in treatment during the last 3 months. 4. HbA1c > 6.5% and< 10% by local laboratory analysis (one retest is permitted with the result of the last test being conclusive). 5. Body Mass Index (BMI) ≥ 18 to ≤ 40.0 kg/m^2. Subjects within the range of >35 to ≤ 40 kg/m^2 need to undergo an ultrasound examination for eligibility. 6. Waist circumference ≤ 45 inches (≤ 114 cm). Subjects within the range of >40 to ≤ 45 inches need to undergo an ultrasound examination for eligibility. 7. Considered generally healthy upon completion of medical history, physical examination and biochemical investigations as judged by the Principal Investigator. 8. Female subjects must be non-pregnant and non-lactating and have a negative serum pregnancy test at Screening. Females may be

	surgically sterile, postmenopausal or of child-bearing potential. Females of childbearing potential must be using an acceptable method of birth control. For specific details please see section 9.1.9 Contraception. 9. Ability to provide written informed consent.
Exclusion Criteria	Subjects who meet any of the following criteria at screening will be excluded from participating in the study: 1. History or current diagnosis with T1DM or T2DM subjects on insulin or other injectable therapies not allowed for this study (as listed in table of Prohibited Medication below). 2. A subject who is already indicated for medication escalation of their current diabetic therapy, or, who based upon study entry criteria, would be indicated for medication escalation during the course of the study (as assessed by the qualified Principal Investigator). 3. A subject who has diabetic complications, i.e., acute proliferative retinopathy or maculopathy, severe gastroparesis, and/or moderate or severe neuropathy, in particular autonomic neuropathy, as judged by the Principal Investigator. 4. Recurrent severe hypoglycemia (more than 1 event ≤ 6 month) or hypoglycemic unawareness or recent severe ketoacidosis (hospitalization ≤ 6 month), as judged by the Principal Investigator. 5. Persistent systolic blood pressure > 150 mm Hg and/or diastolic blood pressure > 95 mm Hg at screening. (Subjects may be re-checked once on the same day). 6. Treatment with antihypertensive medication is not allowed, unless antihypertensive medication is given on a stable dose for at least 2 months prior to screening. 7. Subjects with a clinically significant history or active disease of any of the following body systems: pulmonary, neurological (including dementia, neurodegenerative disease, movement disorder, spinal disorders), pancreatic (including pancreatitis), immunological or systemic inflammatory (including systemic lupus erythematosus [SLE], rheumatoid arthritis [RA]), dermatological, endocrine, genitourinary or hematological (including sickle cell anemia or other anemia syndromes, monocytosis, thrombocytopenia). 8. Subjects with a history or clinically active malignancy (history of basal cell carcinoma [BCC] is allowed). 9. History or current diagnosis of cardiac dysrhythmias or heart disease, defined as symptomatic heart failure (New York Heart Association class III or IV), myocardial infarction, unstable angina requiring medication.

	10. Transient ischemic attack [TIA], cerebral infarct, or cerebral hemorrhage. 11. Invasive cardiovascular procedure, such as coronary artery bypass graft surgery (CABG), or angioplasty/percutaneous coronary intervention (PCI) within 6 months of screening. 12. Presence of clinically significant ECG findings (e.g., QTcF > 450 msec for males, QTcF > 470 msec for females, LBBB) at Screening, or cardiac arrhythmia requiring medical or surgical treatment within 6 months prior to screening. 13. History of renal disease or abnormal kidney function tests at Screening (glomerular filtration rate [GFR] < 60 mL/min/1.73m2 as estimated using the MDRD equation). 14. History or clinically significant active hepatic disease or clinically significant abnormal hepatic function tests at Screening suggestive of hepatic impairment (e.g., ALT and/or AST >2 x ULN), total bilirubin > 1 x ULN). 15. Subjects with a history or presence of any psychiatric disorder that, in the opinion of the Principal Investigator, might confound the results of the trial or pose additional risk in administering the investigational product to the subject. 16. Personal or family history of hypercoagulability or thromboembolic disease, including deep vein thrombosis and/or pulmonary embolism (PE) 17. History of surgical treatment for obesity (bariatric surgery, gastric banding, etc.) or any other gastrointestinal surgery (including appendectomy, cholecystectomy), any malabsorption disorder, severe gastroparesis, any GI procedure for weight loss (including LAP-BAND®), as well as clinically significant gastrointestinal disorders (e.g. peptic ulcers, severe GERD) at Screening. 18. History of any major surgery within 3 months prior to screening. 19. Any nerve stimulation study or implanted stimulator, including previously or currently implanted vagus nerve stimulator, previously or currently implanted spinal cord stimulator, other implanted electronic medical device, such as implanted pacemaker or cardioverter/defibrillator (AICD) or history of seizures. 20. Diagnosis of sleep apnea. 21. Participation in an investigational study within 30 days of screening or 5 half-lives within the last dose of any investigational product given during the investigational study, whichever is longer. 22. Current use of any drugs (other than current treatment for diabetes mellitus) that are known to interfere with glucose or insulin metabolism as stated below in table prohibited medication. 23. Thyroid hormone use is not allowed unless medication is given on a stable dose for at least 3 months prior to screening.

<table>
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24. Chronic use of acetaminophen, and inability to wash-out and abstain from use during the study, as it would interfere with the CGMS assessment.
25. Subject is unable to tolerate adhesive tape or has any unresolved adverse skin reaction in the area of the sensor placement.
26. Implanted pacemaker or cardioverter/defibrillator (AICD).
27. Daily use of more than 5 cigarettes/day or equivalent use of any tobacco- or nicotine-containing product (such as, but not limited to vaping, transdermal nicotine patch, nicotine gum use, etc.) within 4 weeks prior to screening. Subjects must be able to abstain from any tobacco or nicotine containing products during confinement period.
28. Any use of marijuana within 4 weeks prior to screening and positive test at screening.
29. History of any active infection, except mild viral disease, such as common cold, within 30 days prior to screening.
30. History of any recent traumatic injury, including intracerebral hemorrhage and visceral injury.
31. History of alcohol or illicit drug abuse as judged by the Principal Investigator within past 12 months or positive test at screening. Any use of alcohol within 4 days prior to baseline assessment. Subjects must be able to abstain from any alcohol during confinement period.
32. Known history or positive test for hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), human immunodeficiency virus type 1 (HIV-1) or type 2 (HIV-2)
33. Donation or loss of > 500 mL of blood or blood product within 56 days prior to screening
34. Mental incapacity, unwillingness or language barriers precluding adequate understanding and to follow verbal commands during the ultrasound session or cooperation.
35. An abdominal ultrasound scan or exam within 1 month prior to screening and/or any pre-planned ultrasound examinations during the study, or the need to participate in any unplanned outside ultrasound procedures during study.
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<tr><td>Prohibited Medication</td><td>
<table>
<tr><th>Medication or Class</th><th>Indication/Reason</th><th>From time point specified until the end of the study</th></tr>
<tr><td>Antihypertensive medication</td><td>Hypertension</td><td>Excluded unless on stable dose for at least 2 months prior to screening</td></tr>
<tr><td>Lipid lowering drugs that are known to</td><td>Hyperlipidemia</td><td>Must be on a stable dose within 3 months prior to screening</td></tr>
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	interfere with glucose or insulin metabolism		
	Non-prescription drugs/over-the-counter or herbal that are known to interfere with glucose or insulin metabolism		Within one week prior to check-in for first in-house period
	Anti-coagulants (Coumadin, Xarelto)	Thrombotic syndromes, arrhythmias	Within one week of screening
	Analgesics (acetaminophen)	Pain	Within one day of CGMS treatment
	Oral or systemic long-acting corticosteroids, immunosuppressive agents	E.g., chronic or acute non-infectious inflammatory conditions, auto-immune diseases	Within 3 months prior to screening
	Topiramate, monoamine oxidase (MAO) inhibitors, growth hormone.	Use of any drugs that are known to interfere with glucose or insulin metabolism	Within 3 months prior to screening
	Anticholinergic drugs, antispasmodics (e.g., modafinil, phenytoin), 5HT3 antagonists, dopamine antagonists, or opiates, antiemetics, antacids	Reduction/modification of GI motility	Within 2 weeks prior to screening
	Orlistat, lorcaserin, sibutramine, etc., including over-the-counter and herbal supplements, or any medication with a labelled indication for weight loss or gain	Weight control treatment	Within 3 months prior to screening.
	Any anti-diabetic medication, including herbal medicines, OADs, insulin and incretins. (only metformin allowed)	Diabetes	Any prior treatment of insulin within 3 months prior to screening and incretins.
	Selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine	Depression, anxiety, psychiatric disorders	Within 3-month prior to screening.

	reuptake inhibitors (SNRIs), antipsychotics, lithium.		
	Use of SSRIs and SNRIs (including 7bupropion)	For reasons other than active psychiatric indications (e.g., migraine, weight loss, smoking cessation)	Within 3-month prior to screening.
Sample Size	The sample size of 15 subjects was empirically determined and consistent with typical sample sizes used for similar exploratory studies to assess robust data.		
Statistical Methods	Data analyses will follow the statistical analysis plan (SAP). Safety Set: 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 Set: 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. The analysis of the endpoints for Ultrasound Insonification will be summarized descriptively. Safety and tolerability will be assessed based on adverse device effects, tolerability, laboratory parameters, physical examination, vital signs, and ECG parameters throughout the duration of the study. Safety analysis will involve examination of the descriptive statistics and individual subject listings for effects of study treatment on clinical tolerability and safety. Safety data will be summarized using the safety analysis set.		

3.0 LIST OF ABBREVIATIONS

Abbreviation	Definition
ADE	Adverse device effect
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{GIR}	Area under the glucose infusion rate-time curve
BG	Blood glucose
BMI	Body mass index
BUN	Blood urea nitrogen
BW	Body weight
CDM	Clinical data management
C.I.	Confidence interval
CL/F	Apparent clearance
CGMS	Continuous glucose monitoring system
C_{max}	Maximum concentration
CRF	Case report form
CRO	Contract Research Organization
CRP	C-reactive protein
CV	Coefficient of variation
DFT	Deviation from target
DMP	Data management plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic CRF
EGP	Endogenous glucose production
FDA	Food and Drug Administration
FFA	Free fatty acid
FPG	Fasting plasma glucose
FSFV	First subject first visit
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GIR	Glucose infusion rate
GLP-1	Glucagon-like peptide 1
HbA_{1C}	Glycosylated hemoglobin
HBGI	High blood glucose index
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDL	High-density lipoprotein
H-E Clamp	Hyperinsulinemic euglycemic clamp
HIV	Human immunodeficiency virus

HOMA	Homeostasis Model Assessment
HSV	Herpes simplex virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
ICU	Intensive care unit
IL	Interleukin
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous
Kg	Kilogram
lb	Pound
LBGI	Low blood glucose index
LDL	Low-density lipoprotein
LS mean	Least square mean
M	Glucose disposal rate during steady state
Mcg	Microgram
MCR	Glucose metabolic clearance rate during steady state
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
NCA	Non-compartmental analysis
NDA	New Drug Application
Nmol	Nanomol
NPH	Neutral Protamine Hagedorn insulin
NSAID	Non-steroid anti-inflammatory drugs
OAD	Oral antidiabetic drug
OGTT	Oral glucose tolerance test
OTC	Over the counter
PE	Physical examination
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per-Protocol
R_d	Rate of glucose disappearance
rDNA	Recombinant deoxyribonucleic acid
SAP	Statistical analysis plan
SC	Subcutaneous
SI	Insulin Sensitivity Index
SOE	Schedule of events
SUSAR	Suspected unexpected serious adverse reaction
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
$t_{½}$	Terminal insulin half-life

TG	Triglycerides
t_{GIRmax}	Time to maximum glucose infusion rate
T_{max}	Time to maximum serum insulin concentration (in concentration time curve)
TSH	Thyroid-stimulating hormone
U	Unit
UADE	Unanticipated adverse device effect
ULN	Upper limit of normal
US	United States
USP	Unites States Pharmacopeia
VLDL	Very low-density lipoprotein
Vz/F	Apparent volume of distribution at terminal phase
WHO	World Health Organization
YSI	YSI Stat 2300 glucose analyzer

4.0 INTRODUCTION

4.1 Background

Recently, preclinical and clinical studies have been performed on the stimulation of the vagus nerve. Most studies have utilized implanted vagus nerve stimulators (VNS; implanted at the cervical vagus position). Several studies have shown promise in using the stimulator to modulate signaling along neuro-immune pathways/reflexes. Preclinical studies have shown that one such reflex is dependent on a vagus nerve signal that reaches the spleen, alters concentration of norepinephrine and acetylcholine, and modulates the release and production of cytokines by resident macrophages.[1,2] One of the main cytokines under regulation by this reflex is tumor necrosis factor (TNF). Interestingly, TNF is the target of biologic therapies that inhibit TNF signaling in rheumatoid arthritis ([RA] and other chronic inflammatory disease) patients.[3] In RA, symptomatic relief can be achieved in up to 50% of patients using anti-TNF biologics, and recently a first-of-its-kind study has shown that VNS significantly inhibited TNF production for up to 84 days in a pilot study in RA patients (with a concomitant decrease in disease severity as measured by standardized clinical composite scores).[3]

Based on the initial success of using VNS in a bioelectronic medicine (i.e., use of nerve stimulators to effect specific molecular and/or drug target); [2,4] several groups are targeting other (non-splenic) organs, disease applications, and drug targets. In prior literature, the brain-liver connection is well established and includes vagal sensory neural innervation.[5] The central nervous system (CNS) is known to control glucoregulatory response to both hyper- and hypoglycemia.[5-8] However, past VNS experiments measuring effects of metabolic processes have shown complex and contrary results.[9-12] This is in part due to the complexity of the innervation of the liver (i.e., combined afferent and efferent innervation with different function depending on innervation site with the liver), and the inability of implants to stimulate specific neurons (having specific function) within nerves.

Recently, GE has published results on the use of focused, pulsed ultrasound to stimulate small sub-sets of neurons within organs.[13,14] This data includes stimulation of sub-organ sites within the liver (surrounding the porta hepatis and sensory vagal innervation points) and prevention or reversal in several models of hyperglycemia (including LPS-induced hyperglycemia and Fatty Zucker Diabetes/Obesity models). Unlike VNS studies, the precision stimulus has allowed for stimulation of a specific liver-hypothalamic pathway, mapping of the nerve modulation effect, and daily stimulation of the liver site for long term (weeks-month) prevention of diabetes symptoms in several diabetes and obesity models.

For this study, the commercially available ultrasound device, GE General Electric LOGIQ E10 and C16 Probe, will be used to perform hepatic ultrasound insonification in patients with T2DM on metformin monotherapy. This device and probe have previously received IRB approval to perform splenic insonification and measure inflammatory markers from blood samples in healthy human subjects. Additionally, IRB approval has previously been received to perform liver insonification in healthy subjects and subjects with pre-diabetes. In clinical feasibility studies, the system is set to an elastography imaging mode that is utilized within the commercial product for hepatic elastography testing. A pulsed ultrasound stimulus like those utilized in this standard imaging procedure will be applied to the liver (at the porta hepatis region) in this study. Please refer to the User's Manuals for further information on the device and the customized instructions for the pulsed insonification in the elastography setting.

4.2 Rationale for the Proposed Study

The primary outcome to determine whether diagnostic-level ultrasound to the liver has a biological effect, which is measured as a statistically significant change in the primary and secondary markers listed within this protocol (including measures of glucose utilization, glucose tolerance, insulin sensitivity, and glucoregulation).

4.3 Summary of Pre-Clinical /Clinical Studies

A clinical feasibility study has recently been completed that observes the biological effects of ultrasound insonification of the spleen. It is performed in healthy subjects after insonification of the spleen with pulsed ultrasound using the elastography setting within the commercial LOGIQ E10 with C1-6 probe. This study is being conducted to determine if insonification has a biological effect on inflammation markers. This study is being performed based on past GE data showing that the cholinergic anti-inflammatory pathway can be activated by pulsed ultrasound in the spleen.[13]

Additional, pre-clinical studies from GE have demonstrated that pulsed ultrasound focused on specific locations in the liver (i.e. porta hepatis) modifies glucose regulation and insulin sensing and may have beneficial effects in rodent models of diabetes and obesity.[13,14] This feasibility study is designed to test whether insonification at the liver site has a biological effect on measures of glucose utilization, glucose tolerance, insulin sensitivity, and glucoregulation.

4.4 Summary of Known and Potential Risks and Benefits

Although subjects participating in this study will not directly benefit, the knowledge gained may help other people in the future by leading to changes in clinical care. Given the study goal of determining whether insonification at the liver site has a biological effect on measures of glucose utilization, glucose tolerance, insulin sensitivity, and glucoregulation, this study may lead to better methods for treatment.

The General Electric LOGIQ E10 ultrasound pulsed doppler imaging system and C1-6-D XDclear abdominal, curvilinear probe are FDA cleared (K173555) with the indication for

ultrasound evaluation of fetal, abdominal, pediatric, small organ (breast, testes, thyroid), neonatal cephalic, adult cephalic, cardiac (pediatric and adult), peripheral vascular, musculoskeletal conventional and superficial, urology (including prostate), transrectal, transvaginal, transesophageal, and intraoperative (abdominal, thoracic, and vascular).

Since the ultrasound device will be used in this study to achieve organ-specific biological effects, it is being used off-label on this study, and therefore, is considered investigational. Although its use in this study is considered investigational, its use is considered to constitute minimal risk given that it will be operated within the confines of its FDA-cleared equipment parameters and below the guidelines established by the FDA for ultrasound equipment. The FDA guidelines indicate that the derated global maximum acoustic output should not exceed preamendment acoustic output exposure levels. In the case of diagnostic ultrasound, the global maximum derated spatial-peak temporal-average intensity (ISPTA – the highest intensity measured at any point in the ultrasound beam averaged over the pulse repetition period) is ≤ 720 mW/cm^2 with either the global maximum mechanical index being ≤ 1.9 or the global maximum derated spatial-peak pulse-average intensity (ISPPA – the highest intensity measured at any point in the ultrasound beam averaged over the temporal duration of the pulse) being ≤ 190 W/cm^2 (not including ophthalmic use). These FDA guideline values are significantly higher than the maximum spatial-peak temporal-average intensity to be delivered on this study.

The Food and Drug Administration (FDA) has determined that this clinical study is a nonsignificant risk (NSR) device study, because it does not meet the definition of a significant risk (SR device under $812.3(m) of the Investigational Device Exemption (IDE) regulation (21 CFR 812). An NSR study is subject to the abbreviated requirements as described in § 812.2(b) of the IDE regulations.

4.5 Rationale for Treatment and Dose

Insonifications will be performed on three consecutive days (Days 15, 16 and 17) for 15 minutes of ultrasound push-pulse waveform exposure on each day for a total of 45 minutes. Fifteen (15) minutes was chosen as a typical procedure time for imaging, specifically elastography imaging sessions in clinical practice. The 15-minute dose will be targeted to the porta hepatis by the ultrasonographer, and the ultrasonographer may choose to perform breath holds during the procedure in order to locate and target the ultrasound pushpulses on the porta hepatis. If breath holds are utilized during targeting, the subject will be allowed 2-3 minutes between breath holds (and this waiting period will not count toward the 15-minute dose or ultrasound duration).

5.0 STUDY OBJECTIVES AND ENDPOINTS

5.1 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 (H-E 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)

5.2 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 (H-E 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)

5.3 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)

6.0 STUDY DESIGN AND DESCRIPTION

6.1 Study Design

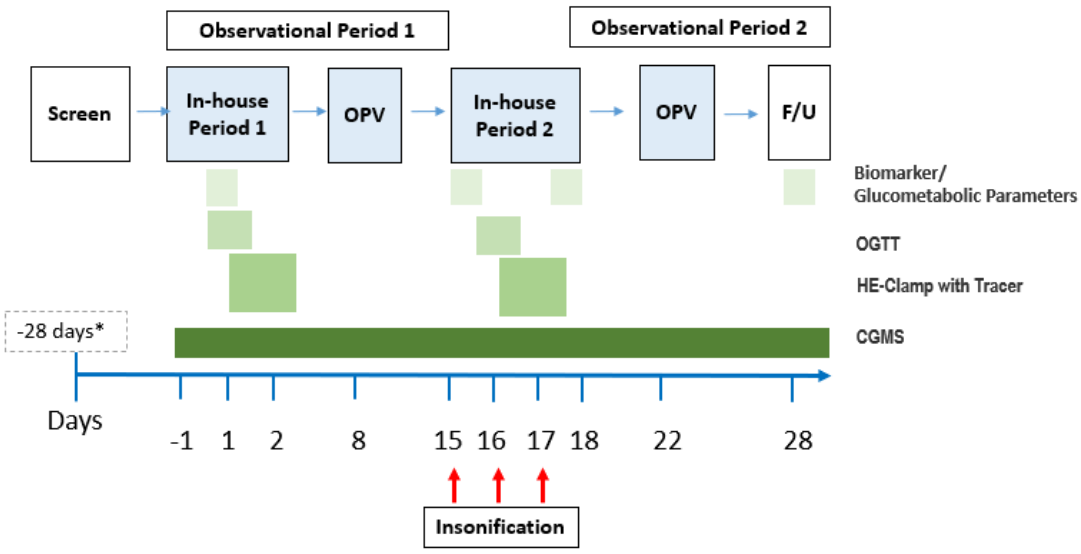
This is an open label, exploratory pilot study to assess the effects of hepatic ultrasound insonification on glucometabolic parameters in subjects with T2DM through selective hepatic ultrasound of the porta hepatis region of the liver utilizing pulsed ultrasound.

Assessments will be performed via CGMS, OGTT, H-E clamp with stable isotope labeled glucose ([6,6-$^{2}H_2$] 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 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/m^2 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.

Figure 6-1 Study Design Schematic



*Screening Period will be extended for at least 30 days, for subjects with BMI> 35.0 and ≤ 40.0 kg/m^2 and/or waist circumference >40 and ≤ 45 inches.

6.2 Study Description

Screening Visit:

At Screening, potential subjects for the study will be provided with written and oral information about the study and the procedures involved. Subjects have to sign the institutional review board (IRB) informed consent form (ICF) prior to entering the study.

The Screening Visit will be performed up to 28 days prior to the first in-house period, to identify eligible subjects for the study. For eligible subjects with a BMI > 35.0 and ≤ 40.0 kg/m^2 and/or waist circumference > 40 and ≤ 45 inches, an ultrasound examination will be added to the screening assessments to confirm eligibility. For these subjects, the screening period will be extended for at least 30 days, starting after the ultrasound examination has been performed. Eligibility per inclusion and exclusion criteria will be re-assessed in a second screening visit prior to the first In-house Period.

All assessments performed at the Screening visit are stated in the Schedule of Events (SOE) and will be recorded in the electronic case report form (eCRF). See section 9.1.2 and 11.2 for further details.

In-house Period 1:

Day -1:

Subjects will check in to the clinic in the morning on Day -1 for a 3-day in-house period.

Check-in assessments will be performed to ensure subjects meet the check-in requirements. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Blood samples will be taken for safety assessments.

Subjects get instructions on maintaining their current diet and physical activity.

Subjects will receive standardized meals throughout their in-house stay. Food intake will be recorded during the entire in-house period by study staff.

Subjects will be connected to a CGMs (e.g., Dexcom G6) and will continue wearing this for the entire study period. Subjects will be blinded to the CGMS data. Monitoring data from Day -1 to Day 14 will be used for the baseline monitoring evaluation. Subjects will be provided with a glucometer. They will be instructed on how to use the glucometer and to monitor their fasting plasma glucose levels daily during the outpatient periods. They will be instructed to contact the study staff, if FPG > 220 mg/dL, they experience symptoms of hypoglycemia, or measures an FPG < 70 mg/dL. Subjects will be frequently contacted by qualified study site staff to review their fasting plasma glucose values.

It will be ensured that subjects have an adequate fasting time prior to the assessments on Day 1.

Day 1:

Following ≥ 10 hour overnight fast, in the morning on Day 1, blood samples will be taken in the fasting state for the assessment of various metabolic parameters and biomarkers, as well as for banking for future exploratory measurements.

Thereafter, at approximately 08:00 h-09:00 h, an OGTT will be performed over 180 min as a baseline assessment. Standardized meals will be provided following the OGTT and in preparation of the clamp, that will be performed on the next day.

At approximately 22:00 h, subjects will receive a low-dose intravenous (IV) infusion of insulin (e.g., NovoLog®) to titrate the BG to a target level of 100 mg/dl (± 10%) in preparation for the H-E clamp. Approximately at midnight, a primed-continuous IV infusion of stable isotope labeled glucose ([6,6-$^{2}H_2$]glucose) will also be initiated for the measurement of EGP.

Day 2:

After completing the fasting EGP assessments, subjects will undergo a 6-hour H-E clamp with stable isotope labeled glucose as baseline assessment. Blood sampling during the clamp will follow time points as indicated in the Clamp Sampling Schedule. Following their glucose clamp, subjects will be monitored post clamp to ensure adequate glycemic stabilization. They will be provided with a standardized meal and may then be released home.

Telephone Visits:

Subjects will be contacted every other day until they come in for the next in-house period, to remind the subjects of their diet /exercise and to check subjects BG.

Outpatient Visit:

Subjects will check-in on Day 8 for their outpatient visit. A sensor change of the CGMS device will be performed. Blood samples will be taken in the fasting state prior to any assessment for the analysis of FPG A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Safety parameters (vital signs, 12-lead ECG, and recording of concomitant medications and adverse device events) and metabolic parameters (body weight/BMI) will be assessed. Subjects will be re-instructed on maintaining their regular diet and physical activity regimes.

In-house Period 2:

Day 15:

Subjects will check in to the clinic in fasting conditions on Day 15 for a 4-day in-house period. Check-in assessments will be performed to ensure subjects meet the check-in requirements. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Fasting blood samples will be taken prior to any assessment for the analysis of safety parameters, various metabolic parameters, as well as for banking for future exploratory measurements.

Subjects will undergo the first of three ultrasound insonifications (following ≥ 10 hour overnight fast). If sonographer is not able to visualize or stimulate target area during the first ultrasound insonification, or a measured distance from the skin surface to the porta hepatis target exceeds 10 cm, the subject will be excluded from the study. Excluded subjects may be replaced. Insonifications will be performed on three consecutive days (Days 15, 16 and 17). Subjects will be insonified by the stimulus pulse for 15 minutes on each day for a total of 45 minutes/study. Breath holds and image targeting will extend the

total duration that the probe is on the subject (without the stimulus). Insonifications will be performed between 07:00 h to 08:30 h.

Subjects will receive standardized meals after the insonification and throughout their in-house stay. Food intake and exercise will be recorded during the entire in-house period by study staff.

Subjects will be re-instructed on maintaining their regular diet and physical activity regimens.

Day 16:

Following ≥ 10 hour overnight fast, in the morning of Day 16, subjects will undergo the second of three ultrasound insonifications. Insonification for each subject will take place at approximately the same time of day as on Day 15.

Thereafter, at approximately 08:00 h-09:00 h, an OGTT will be performed over 180 min. OGTT should start at approximately the same as on Day 1 for the subject. Standardized meals will be provided following the OGTT and in preparation of the clamp, that will be performed on the next day.

Approximately around/after 22:00 h, subjects will receive a low-dose IV infusion of insulin to titrate the BG to a target level of 100 mg/dl (± 10%) in preparation for the H-E clamp. Approximately at midnight, a primed-continuous IV infusion of stable isotope labeled glucose will also be initiated for the measurement of EGP.

Day 17:

Following ≥ 10 hour overnight fast, in the morning of Day 17, subjects will undergo last of three ultrasound insonifications. Insonification for each subject will take place at approximately the same time of day as on Day 15 and Day 16.

Thereafter, fasting EGP assessments will be performed, and subjects will start the 6-hour H-E clamp with stable isotope labeled glucose.

Following the glucose clamp, subjects will be monitored post clamp to ensure adequate glycemic stabilization. Subjects will receive a standardized meal at the end of the procedure.

Day 18:

Blood samples will be taken in the fasting state prior to any assessment for the analysis of FPG, for safety assessments, various metabolic parameters as well as for banking for future exploratory measurements A sensor change of the CGMS device will be performed.

Subjects may be released home or may be asked to stay in-house for an extended time, if deemed appropriate and needed by the Principal Investigator.

Telephone Visits: Subjects will be contacted every other day until the Follow-up Visit, to remind the subjects of their diet /exercise and to check subjects BG.

Outpatient Visit:

Subjects will check-in on Day 22 for their outpatient visit. Blood samples will be taken in the fasting state prior to any assessment for the analysis of FPG. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected. Safety

assessments (vital signs, 12-lead ECG, and recording of concomitant medications and adverse device events) and metabolic parameters (body weight/BMI) will be assessed. Subjects will be re-instructed on maintaining their regular diet and physical activity.

Follow-up Visit:

A Follow-up Visit will be performed on day 28 for final safety procedures, blood sampling will be taken in the fasting state prior to any assessment for the analysis of FPG, for safety, various metabolic parameters as well as for banking for future exploratory measurements. A urine pregnancy test (if applicable), and urine drug screen and alcohol breath test will be collected.

CGM data will be uploaded to site computer via USB transfer from CGM receiver.

Subjects will be instructed to transition back to their regular diabetes medication regimen.

Sampling and Assessment:

Safety assessments will occur throughout the duration of the study, including monitoring of adverse device effects (ADEs), clinical laboratory tests (chemistry, hematology, and urinalysis), vital signs measurements (blood pressure, heart rate, respiration rate, and aural temperature), 12-lead electrocardiograms (ECGs), and physical examinations.

Unscheduled Outpatient Visits:

Additional, unscheduled outpatient visits may occur, in case the CGMS might get dislodged and the sensor would need to be re-inserted by clinic staff.

6.3 Rationale for Study Design and Endpoints

The 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.

Subjects with T2DM will participate in the study, as these subjects are an important target population. These subjects are characterized by relative rather than absolute insulin deficiency in association with variable degrees of insulin resistance.

Subjects on metformin therapy will continue the stable drug dose throughout the entire study.

Subjects will be supplied with a CGMS to ensure that blood glucose levels are monitored closely. CGM as assessment has been chosen to assess the ultrasound insonification effects with a standardized method for efficacy. As assessment of insulin secretion and resistance, an OGTT and H-E clamp will be performed.

Whole-body insulin sensitivity will be assessed with the 2-step H-E clamp procedure. The use of two-stepped IV insulin infusions is used in combination with IV stable isotope labeled glucose infusion to allow the differentiation between hepatic (EGP) and peripheral insulin sensitivity.

Due to the variability in fasting blood glucose concentrations in subjects with diabetes mellitus, which might affect the metabolic response, an overnight baseline period will be

used to establish the target plasma glucose concentration of 100 mg/dL (± 10%) prior to fasting EGP measurements.

Endogenous glucose production will be measured in the fasting state prior to the start of the clamp and suppression of EGP during step 1 of the 2-step H-E clamp. It will be assessed using a stable isotope labeled glucose ([6,6-$^{2}H_2$]glucose) tracer method.

Insulin will be infused at a low dose, followed by a high dose. During the low-dose IV insulin infusion, EGP will be partially suppressed, while during the high dose IV insulin infusion, EGP will be near completely suppressed.

Metabolic changes in response to glucose ingestion will be assessed via an OGTT. It will be performed to assess the postprandial carbohydrate metabolism to see changes in insulin and glucose response.

The other glucometabolic endpoints of this study are chosen to investigate additionalultrasound insonification effects.

6.4 Discontinuation and Stopping Criteria

6.4.1 Criteria for Early Termination of the Study

The study will be completed as planned unless one or more of the following criteria are satisfied that require temporary suspension or early termination of the study.

- New information or other evaluation regarding the safety or efficacy of the study device that indicates a change in the known risk/benefit profile, such that the risk/benefit is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.
- Failure to meet expected enrollment goals
- Administrative Reasons

In the event that the Sponsor, IRB), or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the Sponsor or delegate; the procedure will be followed by the investigational site during the course of termination or study suspension.

6.4.2 Criteria for Early Termination of Individual Subjects

Subjects may discontinue from the study for any of the following reasons:

- Voluntary withdrawal of consent (mandatory removal from study)

Subjects may withdraw their consent to participate in the study at any time.

If a subject withdraws consent, the date and reason for consent withdrawal should be documented. Subjects will be encouraged to remain in the clinic for safety assessments (in case they are currently in one of the in-house periods) until the Principal Investigator deems that it is safe for the subject to be discharged. Subject data will be included in the analysis up to the date of the consent withdrawal.

- ADE or UADE that requires discontinuation at the discretion of the Principal Investigator
- Protocol violation: If protocol violation or concurrent illness occurs, which, in the clinical judgment of the Principal Investigator or after discussion with the Sponsor, may invalidate the study by interfering with the investigation, the subject will be withdrawn by the Principal Investigator.
- Lost to follow-up. The subject did not return to the clinic and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented.
- Discretion of Principal Investigator (document reason on CRF)
- Subject becomes pregnant or begins breastfeeding (mandatory)
- Study discontinuation by Sponsor

Wherever possible, the tests and evaluations, including those listed for the Follow-up Visit should be performed for all subjects who discontinue prior to the completion of the study.

In the event the Principal Investigator determines to terminate a subject participation in the Clinical Study, the Principal Investigator must notify the Sponsor of such decision and rationale immediately in writing. In all cases, the appropriate IRB and other applicable regulatory authorities shall be informed.

7.0 STUDY POPULATION

7.1 Inclusion Criteria

Subjects who meet all the following criteria at Screening to be eligible to participate in the study:

1. Male or female subjects with type 2 diabetes ≥ 12 months.
2. Age ≥ 21 and ≤ 75 years.
3. Stable treatment with diet and exercise or stable treatment with metformin monotherapy. Stable treatment is defined as no change in treatment during the last 3 months.
4. HbA1c > 6.5% and< 10% by local laboratory analysis (one retest is permitted with the result of the last test being conclusive).
5. Body Mass Index (BMI) ≥ 18 to ≤ 40.0 kg/m2. Subjects within the range of >35 to ≤ 40 kg/m^2 need to undergo an ultrasound examination for eligibility.
6. Waist circumference ≤ 45 inches (≤ 114 cm). Subjects within the range of >40 to ≤ 45 inches need to undergo an ultrasound examination for eligibility.
7. Considered generally healthy upon completion of medical history, physical examination and biochemical investigations as judged by the Principal Investigator.
8. Female subjects must be non-pregnant and non-lactating and have a negative serum pregnancy test at Screening. Females may be surgically sterile, postmenopausal or of child-bearing potential. Females of childbearing potential must be using an acceptable method of birth control. For specific details please see section 9.1.9 Contraception.
9. Ability to provide written informed consent.

7.2 Exclusion Criteria

Subjects who meet any of the following criteria at screening will be excluded from participating in the study:

1. History or current diagnosis with T1DM or T2DM subjects on insulin or other injectable therapies not allowed for this study (as listed in table of Prohibited Medication below).
2. A subject who is already indicated for medication escalation of their current diabetic therapy, or, who based upon study entry criteria, would be indicated for medication escalation during the course of the study (as assessed by the qualified Principal Investigator).

3. A subject who has acute proliferative retinopathy or maculopathy, severe gastroparesis, and/or moderate or severe neuropathy, in particular autonomic neuropathy, as judged by the Principal Investigator.
4. Recurrent severe hypoglycemia (more than 1 event ≤ 6 month) or hypoglycemic unawareness or recent severe ketoacidosis (hospitalization ≤ 6 month), as judged by the Principal Investigator.
5. Persistent systolic blood pressure > 150 mm Hg and/or diastolic blood pressure > 95 mm Hg at screening. (Subjects may be re-checked once on the same day).
6. Treatment with antihypertensive medication is not allowed, unless antihypertensive medication is given on a stable dose for at least 2 months prior to screening.
7. Subjects with a clinically significant history or active disease of any of the following body systems: pulmonary, neurological (including dementia, neurodegenerative disease, movement disorder, spinal disorders), pancreatic (including pancreatitis), immunological or systemic inflammatory (including systemic lupus erythematosus [SLE], rheumatoid arthritis [RA]), dermatological, endocrine, genitourinary or hematological (including sickle cell anemia or other anemia syndromes, monocytosis, thrombocytopenia).
8. Subjects with a history or clinically active malignancy (history of basal cell carcinoma [BCC] is allowed).
9. History or current diagnosis of cardiac dysrhythmias or heart disease, defined as symptomatic heart failure (New York Heart Association class III or IV), myocardial infarction, unstable angina requiring medication.
10. Transient ischemic attack [TIA], cerebral infarct, or cerebral hemorrhage.
11. Invasive cardiovascular procedure, such as coronary artery bypass graft surgery (CABG), or angioplasty/percutaneous coronary intervention (PCI) within 6 months of screening.
12. Presence of clinically significant ECG findings (e.g., QTcF > 450 msec for males, QTcF > 470 msec for females, LBBB) at Screening, or cardiac arrhythmia requiring medical or surgical treatment within 6 months prior to screening.
13. History of renal disease or abnormal kidney function tests at Screening (glomerular filtration rate [GFR] < 60 mL/min/1.73m2 as estimated using the MDRD equation).
14. History or active hepatic disease or clinically significant abnormal hepatic function tests at Screening suggestive of hepatic impairment (e.g., ALT and/or AST >2 x ULN), total bilirubin > 1 x ULN).
15. Subjects with a history or presence of any psychiatric disorder that, in the opinion of the Principal Investigator, might confound the results of the trial or pose additional risk in administering the investigational product to the subject.
16. Personal or family history of hypercoagulability or thromboembolic disease, including deep vein thrombosis and/or pulmonary embolism (PE)

17. History of surgical treatment for obesity (bariatric surgery, gastric banding, etc.) or any other gastrointestinal surgery (including appendectomy, cholecystectomy), any malabsorption disorder, severe gastroparesis, any GI procedure for weight loss (including LAP-BAND®), as well as clinically significant gastrointestinal disorders (e.g. peptic ulcers, severe GERD) at Screening.
18. History of any major surgery within 3 months prior to screening.
19. Any nerve stimulation study or implanted stimulator, including previously or currently implanted vagus nerve stimulator, previously or currently implanted spinal cord stimulator, other implanted electronic medical device, such as implanted pacemaker or cardioverter/defibrillator (AICD) or history of seizures.
20. Diagnosis of sleep apnea.
21. Participation in an investigational study within 30 days prior to dosing or 5 half-lives within the last dose of the investigational product given during the investigational study, whichever is longer.
22. Current use of any drugs (other than current treatment for diabetes mellitus) that are known to interfere with glucose or insulin metabolism as stated below in table prohibited medication.
23. Thyroid hormone use is not allowed unless medication is given on a stable dose for at least 3 months prior to screening.
24. Chronic use of acetaminophen, and inability to wash-out and abstain from use during the study, as it would interfere with the CGMS assessment.
25. Subject is unable to tolerate adhesive tape or has any unresolved adverse skin reaction in the area of the sensor placement.
26. Implanted pacemaker or cardioverter/defibrillator (AICD).
27. Daily use of more than 5 cigarettes/day or equivalent use of any tobacco- or nicotine-containing product (such as, but not limited to vaping, transdermal nicotine patch, nicotine gum use, etc.) within 4 weeks prior to screening. Subjects must be able to abstain from any tobacco or nicotine containing products during confinement period.
28. Any use of marijuana within 4 weeks prior to screening and positive test at screening.
29. History of any active infection, except mild viral disease, such as common cold, within 30 days prior to screening.
30. History of any recent traumatic injury, including intracerebral hemorrhage and visceral injury.
31. History of alcohol or illicit drug abuse as judged by the Principal Investigator within past 12 months or positive test at screening. Any use of alcohol within 4 days prior to baseline assessment. Subjects must be able to abstain from any alcohol during confinement period.

32. Known history or positive test for hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), human immunodeficiency virus type 1 (HIV-1) or type 2 (HIV-2)
33. Donation or loss of > 500 mL of blood or blood product within 56 days prior to screening.
34. Mental incapacity, unwillingness or language barriers precluding adequate understanding and to follow verbal commands during the ultrasound session or cooperation.
35. An abdominal ultrasound scan or exam within 1 month prior to screening and/or any pre-planned ultrasound examinations during the study, or the need to participate in any unplanned outside ultrasound procedures during study.

7.3 Prohibited Medications

Use of the agents listed in Table 7-1 (prescription or nonprescription) is prohibited from the time points specified until completion of all study activities.

Table 7-1 Prohibited Medications

Medication or Class	Indication/Reason	From time point specified until the end of the study
Antihypertensive medication	Hypertension	Excluded unless on stable dose for at least 2 months prior to screening
Lipid lowering drugs that are known to interfere with glucose or insulin metabolism	Hyperlipidemia	Must be on a stable dose within 3 months prior to screening
Non-prescription drugs/over-the-counter or herbal that are known to interfere with glucose or insulin metabolism		Within one week prior to check-in for first in-house period
Anti-coagulants (Coumadin, Xarelto)	Thrombotic syndromes, arrhythmias	Within one week of screening
Analgesics (acetaminophen)	Pain	Within one day of CGMS treatment
Oral or systemic long-acting corticosteroids, immunosuppressive agents	E.g., chronic or acute non-infectious inflammatory conditions, auto-immune diseases	Within 3 months prior to screening
Topiramate, monoamine oxidase (MAO) inhibitors, growth hormone.	Use of any drugs that are known to interfere with glucose or insulin metabolism	Within 3 months prior to screening
Anticholinergic drugs, antispasmodics (e.g., modafinil,	Reduction/modification of GI motility	Within 2 weeks prior to screening

phenytoin), 5HT3 antagonists, dopamine antagonists, or opiates, antiemetics, antacids		
Orlistat, lorcaserin, sibutramine, etc., including over-the-counter and herbal supplements, or any medication with a labelled indication for weight loss or gain	Weight control treatment	Within 3 months prior to screening.
Any anti-diabetic medication, including herbal medicines, OADs, insulin and incretins. (only metformin allowed)	Diabetes	Any prior treatment of insulin within 3 months prior to screening and incretins.
Selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), antipsychotics, lithium.	Depression, anxiety, psychiatric disorders	Within 3-month prior to screening.
Use of SSRIs and SNRIs (including bupropion)	For reasons other than active psychiatric indications (e.g., migraine, weight loss, smoking cessation)	Within 3-month prior to screening.

8.0 STUDY MATERIALS AND EQUIPMENT

8.1 Investigational Device

GE LOGIQ™ E10 Imaging Ultrasound System and the C1-6 probe will be used in this study. Device is commercially available. Product is manufactured by General Electric Company, USA.

The equipment necessary for this study is being provided by General Electric to ProSciento as a loan in order to conduct this study. Upon completion of the study, the provided ultrasound equipment will be returned to General Electric. While in possession by ProSciento, the system will be used and maintained in accordance to the terms of the clinical trial agreement (CTA). This includes, but is not limited to, securely maintaining the equipment at ProSciento and only using it for this research study.

For additional information please see the User Guide.

8.2 Storage of Investigational Device

The investigational device must be kept in an appropriate, limited-access, secure location.

The system components will be labeled with the statement "CAUTION - Investigational Device. Limited by United States Law to Investigational Use." The components will also be labeled with the manufacturer name, manufacturer's place of business, packer/distributer (in accordance with 801.1), and the quantity of contents, as appropriate.

Information on each use of the device will be documented, and a device accountability log will be maintained. Information on each component of the system will be provided in the Operations Manual and as available will include labeling, booklet, user manuals, and FDA Letters.

8.3 Packaging, and Labeling of Investigational Products/Device

The Sponsor will provide the Principal Investigator with the device/equipment needed for the insonification in accordance with specific country regulatory requirements. The devices/ equipment will be packaged and shipped to the investigational site after verification of device output pressure by 3rd party (Acertara). The device will be labelled for research use only (including at log-on screen).

8.4 Regimen of Hepatic Ultrasound Insonification

The porta hepatis of the liver, is a deep, short, transverse fissure that is located across the left posterior region of the undersurface of the right lobe of the liver. The porta hepatis encompasses the portal triad (the main portal vein, common hepatic artery, and common bile ducts), lymphatics, nerves, and connective tissue. The portal vein, hepatic artery, and nerves derived from the left vagal trunk and sympathetic plexus ascend the porta hepatis, whereas the common bile duct and lymphatics descend the porta hepatis. The nerves that

run through the porta hepatis are branches of the left vagus nerve and sympathetic branches of the celiac plexus.

Assessment of the porta hepatis region with ultrasound insonifications will be performed by a specialized health care professional, specialized in ultrasonic imaging, with appropriate training in the assessment of the liver and porta hepatis region, as well as in using the GE LOGIQ™ E10 Imaging Ultrasound System. Insonification will be performed on three consecutive days during the study (Days 15, 16 and 17). Subjects will be insonified by the stimulus pulse for 15 minutes on each day, for a total of 45 minutes. The sonographer will perform all ultrasound examinations in all subjects of this study. The sonographer will have the option of performing targeting and insonification during breath holds in order to aid in identification and ultrasound delivery to the target location. If breath holds are utilized, the subject will be allowed 1-2 minutes during each breath hold (during which ultrasound is not being applied), these rest periods will no count toward the 15-minute dose or ultrasound delivery time target.

For the actual assessment, subjects need to be in fasting conditions for ≥10 h prior to assessment. Insonifications will start between approximately 07:00 h and 08:30 h on each insonification day.

For details and images that represent the anatomical area, please see the customized instructions on the insonification and the Operations Manual.

8.5 Ultrasound Insonification Effect

If a study procedure error occurs (including, but not limited to Principal Investigator errors, and/or study subject non-compliance), it should be documented as Protocol Deviation. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional.

Details should be captured on the Dosing Case Report Form. If the subject was exposed to the procedure for a time that exceeds protocol specifications and the subject is symptomatic, then the symptom(s) should be documented as an ADE and be reported.

Should a study procedure error occur, the Principal Investigator or designee should contact the Sponsor or designee within 24 hours.

8.6 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.

8.7 Auxiliary Supply

ProSciento will supply laboratory material necessary for the safety hematology, biochemistry, and urinalyses (incl. pregnancy test) in collaboration with the laboratory.

ProSciento will also provide all necessary material needed for the glucose clamp procedure. Glucose, sodium chloride solutions, insulin, heparin, syringes, and needles used in the clamp will be provided by ProSciento, as well as, standard accessories necessary for an ultrasound examination including ultrasound gel.

9.0 STUDY PLAN

9.1 Study Procedures

9.1.1 Informed Consent and HIPAA Release

Written informed consent will be obtained from each subject prior to performing any study-specific evaluations. The HIPAA release is embedded in the informed consent document. The informed consent document is subject to review and approval by the Sponsor and will be approved by a qualified IRB. The IRB-approved document must contain, at minimum, the eight basic elements of informed consent set forth in applicable law. Only the most recently IRB-approved informed consent document must be used to consent prospective study subjects. The Principal Investigator (according to applicable regulatory requirements), or a person designated by the Principal Investigator and under the Principal Investigator's responsibility, will fully inform the potential study subject of all pertinent aspects of the Clinical Study, including written information given approval/favorable opinion by the IRB.

Prior to the potential subject's participation in the Clinical Study, the written informed consent form must be signed, name filled in and personally dated by the subject and by the person who conducted the informed consent discussion, and by the Principal Investigator. One copy of the signed and dated informed consent document will be given to the subject and the original retained by the Principal Investigator/site.

9.1.2 Screening

Investigators must account for all subjects who sign informed consent forms. The Principal Investigator will keep a Subject Screening and Enrollment Log at the investigational site. Subjects who have screen failed may be allowed to re-screen once at the discretion of the Principal Investigator. A new screening number will be assigned.

Subjects who meet all of the inclusion criteria and none of the exclusion criteria are eligible for this study and will be invited for the in-house periods.

Subjects will be instructed on factors influencing insulin sensitivity. They will be instructed on maintaining their usual diet and exercise programs for the duration of the study and refraining from strenuous exercise prior to the study assessments/check-in to the in-house periods, avoidance of alcohol and herbal products, smoking or medication and illness/infection. Specific factors will be assessed at check-in for the in-house periods.

If subjects are fasting (only water for ≥ 10 hours), all screening assessments may be done on the same day. If subjects are not fasting, they will be invited to return for a second screening visit as available to complete any missing screening procedures (e.g. laboratory assessments) within the available screening window.

For eligible subjects with a BMI> 35.0 and ≤ 40.0 kg/m^2 and/or a waist circumference > 40 and ≤ 45 inches, an ultrasound examination will be added to the screening assessments

to confirm eligibility. Aim is to confirm, that the porta hepatis/region of interest can be viewed, a measured distance from the skin surface to the porta hepatis target does not exceed 10 cm, and it is validated that the subject will be well suited for the insonification procedure during the study. Please see section 9.1.26 Hepatic Ultrasound at Screening for details. If subjects are fasting on the day of the screening visit (only water for ≥ 10 hours), the ultrasound examination may be performed on the same day. If subjects are not fasting, they will be invited to return for a second screening visit as available to complete the missing ultrasound procedure.

If subjects are not eligible per the above stated ultrasound examination, they will be recorded as screen failure. If subjects are eligible per the above stated ultrasound examination, they will continue with the 30-day wait period, starting after the ultrasound examination has been performed, in order to comply with exclusion criterion # 35. Eligibility will be re-assessed on Day -1 based on lab results and examinations that have been performed prior to Day -1, so that all eligibility criteria from screening can be re-assessed on Day -1. Re-assessment can be performed after a 30-day wait period, starting after the ultrasound examination. For details please see the Schedule of Events (SOE).

9.1.3 Demographics and Medical History

Demographic information and medical history, including diabetes and smoking status, and medication history will be obtained at Screening. Subjects who are current regular smokers will not be eligible for inclusion into the study.

9.1.4 Physical Examination

The baseline physical examination will consist of the following body systems: (1) eyes; (2) ears, nose, throat, neck, thyroid; (3) cardiovascular system; (4) respiratory system; (5) gastrointestinal system, mouth; (6) dermatologic system; (7) extremities; (8) musculoskeletal system; (9) central and peripheral nervous system and (10) lymph nodes.

The Toronto Clinical Neuropathy Score will be used to assess the severity of peripheral neuropathy. All aspects of the test, including questionnaire, sensory tests and reflex tests will be performed by a qualified and trained Investigator.

Please see Table 16-3 for the Toronto Clinical Neuropathy Scoring System. The methods for the conduct of the sensory testing will be specified in the Operations Manual.

An abbreviated physical exam (based on symptoms and including examination of cardiovascular, respiratory, gastrointestinal and nervous system) will be performed by the Principal Investigator (or a qualified physician at the investigational site) at time points indicated in the Schedule of Events (SOE). A complete physical examination may be performed in case the subjects have symptoms or at the discretion of the Principal Investigator.

9.1.5 Height, Weight, BMI, and Waist Circumference

Height (without shoes) in meters will be measured once, during the Screening visit.

Weight (without shoes) in kg will be measured fasting in the morning, with light clothing and post void at time points indicated in the Schedule of Events (SOE).

BMI (kg/m^2) will be calculated from height and weight.

Waist circumference (cm) will be measured once, during the Screening, with a tape measure in standing position.

9.1.6 Vital Signs

Vital signs will include body temperature (*aural*), supine blood pressure (after 5 minutes resting), respiration rate and pulse rate (after 5 minutes resting). Vital signs will be measured daily during the in-house period.

On clamp days, vital signs will be assessed pre-clamp and post-clamp procedures.

Vital sign measurements will be performed at days indicated in the Schedule of Events (SOE). Additional measures will be performed as deemed necessary by the Principal Investigator.

9.1.7 Concomitant Illness and Therapy

Concomitant therapy is any medication given in addition to the investigational product (including over-the-counter medications, herbal medications, and vitamin supplements) administered between screening and follow-up.

Concomitant illness is any significant medical condition or disease that is present at study start (signing of informed consent). This includes clinically significant laboratory, electrocardiogram (ECG), or physical examination abnormalities noted at screening examination.

Details of all concomitant illnesses and therapies must be recorded at study entry and must be recorded on the subject's CRF. Any changes in concomitant medication must be recorded at each visit. If the change influences the subject's eligibility to continue in the study, the Sponsor must be informed. The information collected for each concomitant medication includes, at a minimum, start date, stop date or continuing, and indication.

ADEs related to administration of these therapies or procedures must also be documented on the appropriate *e*CRF.

9.1.8 Procedures for Clinical Laboratory Samples

All samples will be collected in accordance with acceptable laboratory procedures. Safety parameter should be taken in the morning after an overnight fast, prior to any pre-planned study procedures.

Laboratory samples will be taken as described in the Schedule of Events (SOE).

Table 9-1 Clinical Laboratory Assessments

Hematology	Serum Chemistry	Urinalysis
CBC with differential: Hematocrit Hemoglobin mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) red cell distribution width (RDW) percentage and absolute differential counts platelet count red cell count (RBC) white blood cell count (WBC)	**Hepatic function panel:** Alanine aminotransferase (ALT/SGPT) albumin, serum alkaline phosphatase, serum aspartate aminotransferase (AST/SGOT) bilirubin, direct (a) bilirubin, total protein, total, serum **Renal function panel:** Albumin, serum BUN BUN: creatinine ratio calcium, serum carbon dioxide, total chloride, serum creatinine, serum glucose, plasma phosphorus, serum potassium, serum sodium, serum **Additional parameters:** Gamma-Glutamyl Transferase Uric Acid	**Routine urinalysis with microscopic examination on positives** (b): Color appearance, specific gravity pH protein glucose ketones occult blood leukocyte esterase nitrite bilirubin urobilinogen

Diagnostic Screening		
Serum/Plasma/Whole Blood	**Urine**	**Breath**
Viral serology: Hepatitis B surface antigen (HBsAg) Hep C antibody (anti-HCV) Anti-HIV-1 Anti-HIV-2 **Thyroid-stimulating hormone** (TSH) (c) **Coagulation:** Partial thromboplastin time (PTT) PT International normalized ratio (INR) **Lipid profile:** Cholesterol, total	**Drug Screen Profile** Urine drug screen (12 panel) via commercial kit at the site. Urine drug screen at screening, at check-in for each day -1 via commercial kit at the investigational site. **Female Subjects Only** Human chorionic gonadotropin (hCG) performed at Screening. Follicle-stimulating hormone (FSH) test for postmenopausal women (defined as amenorrheic female subjects <60 years of age and not surgically sterile) at Screening.	Alcohol breath test at timepoints stated in the SOE at the investigational site

HDL-C (Cholesterol) LDL-C VLDL-C Triglycerides FFA	Urine pregnancy testing via commercial kit at the site at timepoints stated in the Schedule of Events.
Glucose Metabolism Parameter: Fasting Plasma Glucose (FPG) HbA1c Fructosamine HOMA-IR HOMA-B	
Fasting Insulin **Fasting C-Peptide**	
Incretins/exploratory biomarkers: Total GLP-1 Glucagon Leptin Ghrelin	
Inflammatory biomarker: Cytokines (IL-6) Adiponectin CRP	

(a) Assess only if total bilirubin ≥2.0 mg/dL.
(b) Microscopic analysis should be performed only if urine evaluations are abnormal.
(c) In the event of abnormal TSH, Free T3/T4 may be collected at Principal Investigator discretion.

The responsible laboratories will perform all assigned laboratory tests, e.g., listed above. Specialty labs may be responsible for specific laboratory parameters. The results of laboratory tests will be sent to the Principal Investigator or designee, who is responsible for reviewing these results. All laboratory safety data will be faxed or transferred electronically.

Laboratory reports must be signed and dated by the Principal Investigator or designee indicating that the report has been reviewed and any abnormalities have been assessed for clinical significance.

All clinically significant laboratory abnormalities must be recorded as an ADE. A clinically significant laboratory abnormality may be verified by retesting and may be followed upon discretion of the Principal Investigator.

9.1.9 Contraception

Female subjects must be non-pregnant and non-lactating.

Females may be either surgically sterile (e.g., bilateral tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) or post-menopausal for >12 months without alternative medical cause. The site will make an effort to retrieve medical records to document the sterility, however, the absence of records will not exclude the subject. In the event that medical records cannot be obtained, serum pregnancy testing will be conducted at Screening, and urine pregnancy testing will be conducted throughout the study. Postmenopausal status will be confirmed through testing of FSH levels outside the norm based on specific lab at screening for amenorrheic female subjects < 60 years of age.

Only female subjects of childbearing potential must use highly effective contraceptive methods, that are considered those with failure rate less than 1% undesired pregnancies per year "with perfect use", including hormonal intrauterine devices (coil), oral hormonal contraceptives, sexual abstinence, or a surgically sterilized partner. Subjects on hormonal contraception must be on stable hormonal contraception for >2 month prior to Screening and for the entire study.

Male subjects are not required to use any methods of contraception.

9.1.10 Pregnancy

In the event a subject becomes pregnant during the study, she should be withdrawn. However, the subject will be encouraged to complete the post-treatment follow-up portion of the study to the extent that study procedures do not interfere with the pregnancy.

If the pregnancy occurs at any time during the study, the pregnancy should be reported immediately to the Sponsor, using a pregnancy notification form.

Study subjects will give consent on enrollment that the Principal Investigator will report any pregnancy during the study to the Sponsor and that they will be asked to provide information about her pregnancy, delivery, and the health of her infant until age one month. Payment for all aspects of obstetrical care, child, or related care will be the subject's responsibility.

All reported pregnancies will be followed up to final outcome, using the pregnancy and pregnancy follow-up forms. The outcome, including any premature termination, will be reported to the Sponsor. An evaluation after the birth of the child may also be conducted.

Study-related pregnancy complications must be recorded as adverse event(s).

9.1.11 ECG Procedure

A standard 12-lead ECG will be recorded after 5 minutes in a supine position.

The Principal Investigator (or designee) will interpret the ECG's by use of an electronic measurement using the following categories: within normal limits, abnormal but not clinically significant, or abnormal with clinical significance. ECGs are performed according to the Schedule of Events (SOE). The following parameters will be recorded

from the subject's ECG trace as calculated by the machine algorithm: heart rate, QT interval, PR interval, QRS interval, RR interval, and QTc (corrected) using the Fridericia correction (QTcF = QT ÷ cube root of the R-R interval [where R-R is the duration of the entire cardiac cycle]).

When ECGs are to be collected at the same time point as a blood collection, ECGs should be collected first to avoid any artificially increased heart rates due to the blood collection.

ECGs on clamp days will be performed before and after the clamp procedure.

In some cases, it may be appropriate to repeat abnormal ECGs. If a machine-read QTc value is prolonged, repeat measurements may not be necessary if the Principal Investigator's interpretation determines that the QTc value is in the acceptable range.

9.1.12 Check-in Procedure

All subjects will check in to the clinic in the morning to the In-house Periods. The following will be assessed:

1. Alcohol breath test.
2. Urine drug screen.
3. Urine pregnancy test.
4. Any use of prescription or non-prescription medicine/herbal medicine other than the allowed concomitant medications.
5. Any medical condition that could interfere with glucose metabolism, as judged by the Principal Investigator.
6. Any vigorous exercise 24 hours prior to check-in to the in-house periods (for example: heavy lifting, weight training, calisthenics, and aerobic activity).

Subjects who fulfill one or more of the stated criteria at check-in, will not be able to continue onto the in-house period. The in-house period will be rescheduled. Each in-house period may be rescheduled no more than once. Subjects will be given up to 5 days to reschedule check-in on Day 15, in case of a weekend/holiday or subject's personal issue. However, once checked-in for Day 15, there will not be a window to reschedule remaining insonification procedures (Day 16 & 17). After that, the subject will be excluded from the study. Replacement of subjects is be permitted.

9.1.13 Fasting Plasma Glucose

Fasting plasma glucose will be measured during the in-clinic period with an YSI 2300 STAT glucose analyzer (YSI) at time points stated in the Schedule of Events (SOE). On the days that subjects are treated for hypoglycemic events during the evening hours, the morning lab will not be in the fasting state.

9.1.14 Self-Monitoring of Blood Glucose (SMBG)

Subjects will also be provided with a SMBG device for glucose monitoring. They will be instructed on how to use the glucometer and to monitor their fasting plasma glucose levels. For further details on capturing data and documentation, please see the Operations Manual.

In this study, a BG meter (e.g., Accu-Check® Aviva Plus*)* will be used, so that data can be downloaded by the site at the study visits. A confirmation measurement of the glucose values may be performed by a standard glucose analyzer.

Although subjects apply whole blood to the test strip, the system has been calibrated to deliver plasma-like values for easier comparison to laboratory results. The term plasma glucose will be used throughout the protocol in reference to the glucometer results, while the term blood glucose will be used in reference to the blood sample that is used for measurement.

Subjects will be instructed to perform a SMBG check every morning in fasting condition (FPG) during the outpatient period.

Additional blood glucose measurements can be performed by fingerstick at any time for safety reason, or when a CGMS measurement needs to be confirmed or validated. Subjects will be instructed to monitor their fasting plasma glucose levels daily during the outpatient period. Subjects will be frequently contacted by qualified study site staff via telephone calls to review their fasting plasma glucose values. These phone calls/phone visits will be documented in a Telephone Log. For further details please see the Operations Manual.

Subjects are instructed to contact study staff if they measure an FPG (after 10 hours of fasting) > 220 mg/dL, experiences symptoms of hypoglycemia, or measures an FPG < 70 mg/dL. A confirmation measurement of the glucose values may be performed by a laboratory method (YSI). In case FPG value have reached the stated limits, and values are confirmed by a standard glucose analyzer, subjects will be counseled on how to keep their FPG within these limits. The subject will then have a reassessment, per PI discretion, of their FPG, confirmed by the glucose analyzer. If the retested and verified FPG is still outside the stated thresholds they will be dropped from the study and may be replaced. At the end of the study, subjects may keep their glucometer to measure to control their blood glucose at home.

9.1.15 Standardized Meals

During in-house period(s), subjects will receive standardized meals. These meals will be provided up to 12 hours prior to OGTT and start of scheduled clamp procedures. Meals will be provided after the OGTT and or clamp procedure have been terminated.

The standardized weight maintaining meals will be provided using estimated BMR × activity factor of 1.5 to determine daily caloric intake. Macronutrient composition will be standardized.

9.1.16 Dietary and Nutrition Counseling

Subjects will be counseled to maintain their normal diet and exercise regimen. They will be instructed not to start any new diets, supplements, or exercise programs during the study.

9.1.17 Physical Activity

Subjects will be instructed to maintain their usual exercise programs for the duration of the study. Subjects will be asked to refrain from vigorous exercise 24 hours prior to check-in to the in-house periods and during in-house stays (for example: heavy lifting, weight training, calisthenics, and aerobic activity).

9.1.18 CGMS

All subjects will be connected to a CGMS (e.g., Dexcom G6, San Diego, CA, USA) to register blood glucose concentrations during the study as described in the Operations Manual.

Please refer to the User Guide for all detailed device instructions and the Operations Manual.

In case the CGMS might get dislodged and the sensor would need to be re-inserted, subjects would be asked to come to the clinic.

9.1.19 Oral Glucose Tolerance Test (OGTT)

An oral glucose tolerance test (OGTT) will be administered at the time points specified in the Schedule of Events (SOE).

Subjects will be fasted ≥ 10 hours prior to the OGTT. At approximately 08:00 h-09:00 h the OGTT will begin. Subjects will be asked to drink 75 g of glucose in a 10-fluid ounce beverage over 5 minutes. The glucose ingestion will be followed by approximately 100 mL of water. The subject will rest for the duration of test and will abstain from eating. Only small amounts of water may be consumed for the duration of the test. Sampling for fasting glucose, C-peptide, insulin sampling will be performed before the OGTT and 15, 30, 60, 90, 120, 150 and 180 min after the start of the glucose administration.

The time of all blood draws and the time when the subjects start to drink the glucose solution will be recorded.

OGTT sampling schedule: Time points are relative to start of OGTT procedure. All samples will be taken within a window of ± 3-minutes.

All OGTT procedures during the in-house periods on Day 1 and Day 16 will follow the same schedule.

The OGTT procedure for each subject (inter-individual) will start one hour (nominal time) after the start of their hepatic ultrasound insonification. A window of ± 15 minutes will be allowed.

Insonification and OGTT on Days 1 and 16 should start at the same time (actual time) for each subject (intra-individual).

Table 9-2 Sampling Schedule for OGTT

Nominal time (min) relative to Start of OGTT	**-15**	**0**	**15**	**30**	**60**	**90**	**120**	**150**	**180**
Standard Test Solution (75 g dextrose)		**X**							
Plasma Glucose	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Insulin and C-peptide	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**

9.1.20 Overnight IV Insulin Infusion

At approximately 22:00 pm on the evening prior to a clamping procedure, an overnight IV insulin infusion to achieve plasma glucose levels of approximately 100 ± 10 mg/dL during the last two hours of the overnight IV infusion prior to the fasting EGP assessment period. Glucose levels will be monitored frequently.

On the evening prior to the clamp procedure, subjects will receive a standardized dinner, which will be followed by an approximately 10-hour overnight fast.

The aim is to only use insulin to achieve the target glucose level and to avoid simultaneous infusion of glucose. In case blood glucose levels are lower than the target level, an IV glucose infusion may be used and recorded.

The amount of insulin administered will be recorded and expressed in $mU/m^2/min$ for each individual subject considering his/her body surface area. GIR will be recorded during the entire clamp procedure.

For details please see the Operations Manual.

9.1.21 Intravenous Infusion of Stable Isotope Labeled Glucose and Assessment of Endogenous Glucose Production

Endogenous glucose production or EGP will be measured in the fasting state prior to the start of the clamp and during the 2-step H-E clamp procedure to assess insulin-induced suppression of EGP using a stable isotope labeled glucose ([6,6-$^2H_2$]glucose) tracer method.

A primed-continuous IV infusion of [6,6-$^2H_2$]glucose will be initiated prior to the clamp and continued throughout the clamp at various rates. At approximately midnight, 5.5 mg [6,6-$^2H_2$]glucose/kg body weight will be injected (priming dose) over 10 minutes, followed by a constant infusion of 0.05 mg [6,6-$^2H_2$]glucose/kg body weight/min until the end of the fasting EGP assessment period, followed by a rate of 0.025 mg [6,6-$^2H_2$] glucose/kg body weight/min from beginning to end of the Step 1 insulin infusion. The [6,6-$^2H_2$]glucose infusion is discontinued at the end of the Step 1 of the clamp. Blood samples will be taken during the last 30 minutes of the fasting, step 1 and step 2 periods (see Table 9-3). The glucose enrichment in the plasma will be measured using gas chromatography-mass spectrometry (GC-MS). EGP will be calculated during the last 30 minutes of each assessment period from the dilution of the infused stable isotope labeled glucose using the steady-state method of Steele.

For details please see the Operations Manual and Pharmacy Manual.

9.1.22 Two-step Hyperinsulinemic, Euglycemic Clamp

On the evening prior to the clamp procedure, subjects will receive a standardized dinner, which will be followed by an approximately 10-hour overnight fast.

Subjects will receive an overnight insulin IV infusion to achieve plasma glucose levels of approximately 100 ± 10 mg/dL during the last two hours of the overnight IV infusion prior to the fasting EGP assessment period.

Subjects will be connected to the Biostator prior to the start of the clamp procedure.

During step 1 and step 2 of the clamp procedure, insulin will be infused at two infusion rates each lasting approximately 180 minutes and D20W will be enriched with stable isotope labeled glucose to achieve a stable isotope glucose enrichment comparable to that of plasma in the fasting state (e.g., 2.5 to 3% for plasma [6,6-$^2H_2$]glucose). Insulin will be infused IV by means of a precision pump for 180 min at the following rate:

Step 1: For the Step 1 insulin infusion of the clamp procedures at Day 2, an amount of 10 mU/m^2/min will be added to the average insulin infusion rate which was provided per algorithm over the last 2 hours of the overnight infusion. This will be calculated for each individual subject.

For the Step 1 insulin infusion of the clamp procedures at Day 17, the insulin infusion rate should be identical to the rate used at Day 2. It is important that each subject receives the same insulin infusion rate on Day 17 that he/she received on Day 2.

Step 2: After 180 minutes (end of Step 1), the insulin infusion will be increased to 120 mU/m^2/min for a further 180 minutes (Step 2) of the clamp for all subjects at Day 2 and Day 17. This will provide supraphysiological insulin concentrations that will near-maximally stimulate glucose disposal. EGP is expected to be near maximally suppressed.

The glucose infusion rate (GIR; 20% v/v) required to maintain arterialized venous blood glucose (glucose oxidase method) at the target level of 100 ± 10 mg/dL will be recorded throughout the clamp. 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.

Insulin infusion rates may be adjusted per PI discretion and discussion.

The clamp procedure will terminate approximately 6 hours after the initiation of Step 1. The insulin infusion will be discontinued after the last blood sample has been collected.

As a background validation with a second methodology for safety, the standardized measurements of the Biostator will be verified on a regular basis using the YSI reference device at approximately every 30 minutes.

All clamp procedures will follow the same schedule.

For details please see the Operations Manual.

Figure 9-2 Schematic of Clamp Procedure

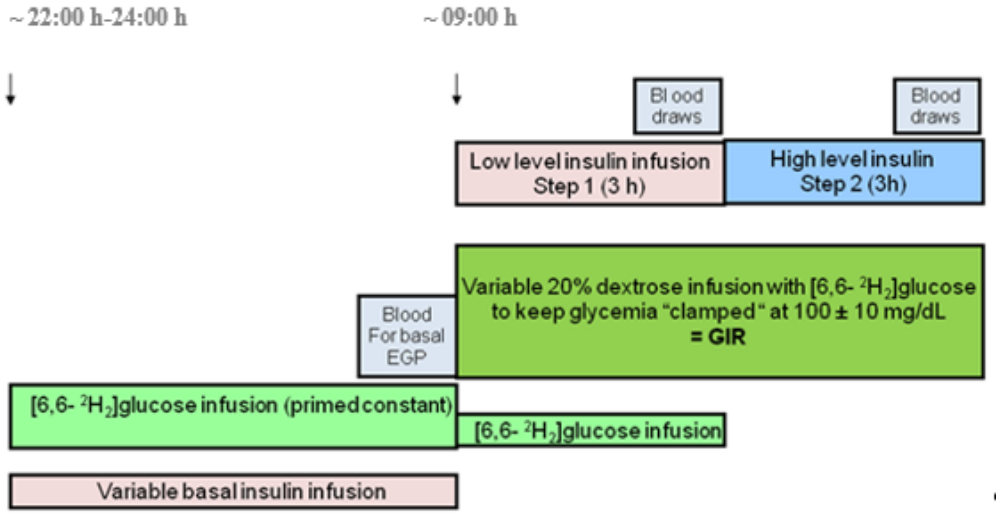


Table 9-3 Schedule for Clamp and Tracer

Parameter		**Time in Hours of Day (time is approximate)**												
	22:00	24:00	8:30	8:40	8:50	9:00	11:30	11:40	11:50	12:00	14:30	14:40	14:50	15:00
Primed Continuous [6,6-$^2H_2$] Glucose Infusion		X	→	→	→	→	→	→	→	X				
Overnight Insulin Infusion Glucose Control	X	X	→	→	→	X								
Step 1 Insulin Infusion (low dose)						X	→	→	→	X				
Step 2 Insulin Infusion (high dose)										X	→	→	→	X
Dextrose 20% Infusion Enriched With [6,6-$^2H_2$] Glucose						X	→	→	→	→	→	→	→	X
Plasma [6,6-$^2H_2$] Glucose Enrichment	X*		X	X	X	X	X	X	X	X	X	X	X	X
Glucose			X	X	X	X	X	X	X	X	X	X	X	X
Insulin			X	X	X	X	X	X	X	X	X	X	X	X
FFA/Triglycerides			X	X	X	X	X	X	X	X	X	X	X	X

*Sample may be taken within window of 22:00 h and 24:00 h. It is of importance, that the sample is taken prior to the start of the [6,6-^{2}H2]glucose infusion.

9.1.23 Assessments of Ultrasound Insonification Effects

Blood for analyses for the assessment of ultrasound insonification effects will be collected at the time points indicated in the Schedule of Events (SOE). Sampling for exploratory biomarkers such as incretins/ metabolic hormones (total GLP-1, Ghrelin, Glucagon, Leptin), glucose parameters (HbA1c, Fructosamine), lipid metabolism parameters (total cholesterol, LDL-C, HDL-C, VLDL-C, Triglycerides, Free Fatty Acids), as well as inflammatory biomarkers (Cytokines [IL-6], Adiponectin, CRP) will be collected, and analyzed or stored for future analysis.

9.1.24 Hepatic Ultrasound Insonification

Patients should report if they need/participate in any outside ultrasound procedures during study.

All three insonifications will be performed after an overnight fast (no food or drinks except for water for at least 10 hours) at approximately the same time on each day. Insonification will start at approximately 07:00 h each day. If multiple subjects are scheduled for insonification on one day, insonification may be staggered and performed between 07:00 h and 08:30 h on that day. Clock time of insonification will be recorded and the same subjects (intra-individual) will undergo their insonification at approximately the same time (actual time) on each day. A window of ± 15 minutes will be allowed.

Insonifications will be performed prior to any other assessment, such as OGTT or start of H-E clamp.

Check of Imaging Criteria on Day 15:

If sonographer is not able to visualize or stimulate target area on ultrasound, or a measured distance from the skin surface to the porta hepatis target exceeds 10 cm, subjects will be excluded from the study and may be replaced.

For details on the hepatic ultrasound insonification, image saving, data recording and data transfer, please see the Operations Manual and the customized instructions on pulsed insonification in the elastography setting.

9.1.25 Blood Volume

Total blood sampling volume for subjects will be stated in the Laboratory Manual for this study.

9.1.26 Hepatic Ultrasound at Screening

Only eligible subjects with a BMI > 35.0 and ≤ 40.0 kg/m^2 and/or waist circumference > 40 and ≤ 45 inches will need to undergo this ultrasound examination at Screening.

Aim is to confirm, that the porta hepatis/region of interest can be viewed, the measured distance from the skin surface to the porta hepatis target does not exceed 10 cm, and it is

validated that the subject will be well suited for the insonification procedure during the study.

The ultrasound examination will be performed after an overnight fast (no food or drinks except for water for at least 10 hours). If sonographer is not able to visualize the target area on ultrasound, or the measured distance from the skin surface to the porta hepatis target exceeds 10 cm, subjects will be excluded from the study and may be replaced.

10.0 FOR DETAILS ON THE HEPATIC ULTRASOUND, IMAGE SAVING, DATA RECORDING AND DATA TRANSFER, PLEASE SEE THE OPERATIONS MANUAL. ADVERSE DEVICE EFFECTS

10.1 Definitions

10.1.1 Adverse Device Effect (ADE)

An ADE is any undesirable and unintended medical event occurring to a subject in a clinical study, whether or not related to the study procedures. This includes events from the first study related activity after the subject has signed the informed consent and until post treatment follow-up period as defined in the protocol. The following should not be recorded as ADEs, if recorded as medical history/concomitant illness on the CRF at screening:

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first study related activity after the subject has signed the informed consent
- Pre-existing conditions found as a result of screening procedures
- Pre-existing events that has not worsened in intensity or frequency from baseline

10.1.2 Clinical Laboratory Adverse Device Event

A clinical laboratory ADE is any clinically significant laboratory abnormality that suggests a disease and/or organ toxicity and is of a severity, which requires active management, (i.e. more frequent follow-up or diagnostic investigation).

A laboratory re-test and/or continued monitoring of an abnormal value is not considered an intervention. In addition, repeated or additional noninvasive testing for verification, evaluation or monitoring of an abnormality is not considered an intervention.

10.1.3 Unanticipated Adverse Device Effect (UADE)

Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application) or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

10.1.4 Severity of ADEs

ADEs will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v5.0). The different categories of intensity (severity) are characterized as follows:

Grade 1	Mild; asymptomatic, or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

Activities of Daily Living (ADL)
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

10.1.5 Relationship to Study Treatment

The relationship of each ADE to the study procedure(s) will be assessed by the Principal Investigator or Sub-Investigator on the basis of his/her clinical judgment and the following definitions:

1= **Related**:
The ADE follows a reasonable temporal sequence from the exposure to the study procedures, and cannot be reasonably explained by the subject's clinical state or other factors (e.g., disease under study, concurrent diseases, or concomitant medications).

The ADE follows a reasonable temporal sequence from exposure to the study procedures, and represents a known reaction to the study treatments or procedures, or is predicted by the known pharmacological properties of the treatment.

The ADE resolves with discontinuation of the investigational product and/or recurs with rechallenge, if applicable.

2 = **Not Related**:
The ADE does not follow a reasonable temporal sequence from exposure to the study procedures, or can be reasonably explained by the subject's clinical state or other factors (e.g., disease under study, concurrent diseases and concomitant medications).

10.2 Procedures

10.2.1 Collection and Recording of ADEs

Collection of all ADEs (UADEs and ADEs) will commence from the time the subject signs the informed consent to participate in the study until the post-treatment follow-up

visit. At each study visit and throughout the in-house treatment period, the Principal Investigator will assess whether any subjective ADEs have occurred. In order to avoid bias in eliciting ADEs, a non-specific question, such as "How have you been feeling since your last visit?" may be asked. Subjects may report ADEs occurring at any other time during the study.

All subjects experiencing ADEs must be monitored and given appropriate medical treatment at the discretion and judgement of the Principal Investigator until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or until there is a satisfactory explanation for the changes observed. All ADEs will be documented in the ADE page of the eCRF, whether or not the Principal Investigator concludes that the event is related to the treatment/procedure. The event term, start and stop dates, severity, action taken and outcome, will be documented.

10.2.2 Collection and Reporting of UADEs

When an UADE occurs, the Principal Investigator should report it according to the following procedure:

An UADE form must be completed immediately or within 24 hours of first onset or notification of the event. The information should be completed as fully as possible but contain, at a minimum a short description of the event and the reason why the event is categorized as serious, subject identification number, Principal Investigator's name, name of the study procedure and a causality assessment.

In the interest of subject safety, and in order to fulfill regulatory requirements**,** all UADEs should be reported to the IRB and Sponsor or a designated qualified vendor within 24 hours of the Study Center's first knowledge of the event, but in no event later than 10 working days.

The collection of UADEs will begin after the subject signs the informed consent form and stop at the end of the subject's follow-up period. An Initial UADE Form should be completed, and a copy should be securely faxed or e-mailed to the Sponsor or designated qualified vendor.

10.3 Anticipated ADEs

Normal precautions taken for a human study, including the provision of emergency equipment, will be taken during this study. Qualified and well-trained physicians and medical staff will instruct the subjects.

Possible anticipated, adverse effects or inconveniences for subjects participating in the study procedure are:

10.3.1 Hypoglycemia

There is a small risk of severe hypoglycemia (requiring assistance of the study staff) when an exogenous insulin analog is administered. The glucose clamp will be performed

under continuous surveillance and frequent monitoring of blood glucose levels as specified. Since there is simultaneous IV infusion of glucose, the occurrence of severe hypoglycemia is unlikely. If a blood glucose value $\leq$ 70 mg/dL is recorded and confirmed by YSI, or the subject has neurogenic symptoms (palpitations, tremor, hunger, sweats) or symptoms of neuroglycopenia (confusion, lethargy, agitation, reduced level of consciousness), the subject must be treated at the discretion of the Principal Investigator to prevent or alleviate neuroglycopenic symptoms. Treatment may include ingestion of carbohydrates (e.g., apple juice), IV glucose bolus injections, IV glucose infusions or other methods as required per Principal Investigator. Subjects will be monitored closely, and it will be a clinical decision made by the Principal Investigator to move from oral to IV glucose bolus, to IV glucose infusion. Severe hypoglycemia (as defined by the ADA Workgroup) should be treated at the Principal Investigator's discretion according to best available medical practice. As a safety precaution, a glucose solution will be available near the subject.

Hypoglycemic events will be recorded as adverse events, if symptoms develop or if blood glucose measured is $\leq$ 70 mg/dL, even if no symptoms are present. The subjects will only be discharged from the clinic, if deemed safe by the Principal Investigator.

10.3.2 Procedure Related Adverse Events

Study procedures involve the placement of an IV catheter which may lead to allergic reaction, redness, swelling, bruising, pain, bleeding or infection at catheter insertion site.

The study procedures also involved the use of adhesive to secure the placement medical equipment. The adhesive may cause an allergic reaction, redness, swelling or itching when in contact with the skin.

10.3.3 Risks Related to CGMS

During the study, CGMS will be used. The CGM poses no major risks to the subjects. CGMS sensor placement may lead to mild skin irritation at the insertion site, such as erythema, edema, rash, bleeding, itching, bruising, scaling skin, and induration. Rarely sensors may fracture, and a small piece may remain under the skin which will need to be removed by a study clinician. This may cause mild discomfort, bruising, or temporary bleeding. In order to avoid risks, CGM catheter will be placed under sterile conditions by experienced staff members. In the case of signs of inflammation or bruising at the sensor insertion site, the sensor will be removed immediately, and standard of care will apply. The subjects will be given one of the study physician or nurse practitioner's cell phone number to contact for any questions, concerns or problems.

10.3.4 Risks Related to OGTT

Subjects will participate in OGTTs during the study, which may lead to nausea or gastrointestinal discomfort following the drink. Subjects will be asked to lie flat and rest as typically these feelings pass very quickly. If the nausea does not pass, then the study will be terminated and they will be offered food to eat, which will mitigate the problem.

10.3.5 Risks Related to Repeated Blood Draws

Subjects will participate in several blood draws throughout the course of the study which have the potential to cause a venous line-vasovagal response, bruising, tenderness, and rarely infection. Treatment of any complications, if necessary, usually involves warm compresses to the area for one to several days. Although the study involves numerous blood draws, it is considered minimal risk since the study will only involve healthy adult individuals. In addition, the overall blood volume that will be collected, will be below the maximum blood collection volume deemed minimal risk (i.e., no more than 550 ml in an 8-week period).

10.3.6 Risk Related to Hepatic Insonification

The General Electric LOGIQ E10 ultrasound pulsed doppler imaging system and C1-6-D XDclear abdominal curvilinear probe are FDA cleared (K173555) with the indication for ultrasound evaluation of fetal, abdominal, pediatric, small organ (breast, testes, thyroid), neonatal cephalic, adult cephalic, cardiac (pediatric and adult), peripheral vascular, musculoskeletal conventional and superficial, urology (including prostate), transrectal, transvaginal, transesophageal, and intraoperative (abdominal, thoracic, and vascular).

Since the ultrasound device will be used on this study to test for organ-specific biological effects, it is being used off-label on this study, and therefore, is considered investigational. Although its use in this study is considered investigational, its use is considered to constitute minimal risk given that it will be operated within the confines of its FDA-cleared equipment parameters and below the guidelines established by the FDA for ultrasound equipment. The FDA guidelines indicate that the derated global maximum acoustic output should not exceed preamendment acoustic output exposure levels. In the case of diagnostic ultrasound, the global maximum derated spatial-peak temporal-average intensity (ISPTA – the highest intensity measured at any point in the ultrasound beam averaged over the pulse repetition period) is ≤ 720 mW/cm^2 with either the global maximum mechanical index being ≤ 1.9 or the global maximum derated spatial-peak pulse-average intensity (ISPPA – the highest intensity measured at any point in the ultrasound beam averaged over the temporal duration of the pulse) being ≤ 190 W/cm^2 (not including ophthalmic use).

10.4 Follow-up of ADEs and UADEs

All ADEs should be followed up and subjects will be rendered appropriate medical care and treatment at the discretion of the Principal Investigator until resolution or until the Principal Investigator and Sponsor concludes that “further follow-up is not necessary”. If the ADE has not resolved by the post-treatment follow-up visit, the stop date will be recorded as “ongoing.”

All UADEs should be followed up until resolution or permanent outcome of the event or until the Principal Investigator and Sponsor judge that further follow-up is not necessary.

If information is not available at the time of the first report and becomes available at a later date, the Principal Investigator should complete a follow-up UADE form at the earliest possible or provide other written documentation and send it electronically within 24 hours of receipt of information to the Sponsor or designee. Copies of any relevant data from the hospital notes (e.g., ECGs, laboratory tests, discharge summary, postmortem results) should be sent accordingly.

All other non-serious ADEs must be followed until the outcome of the event is "recovering" (for chronic conditions), or "recovered", or until the end of the post-treatment follow-up stated in the protocol, whichever comes first, and until all queries related to these ADE's have been resolved.

10.4.1 Safety Reporting to IRBs and Regulatory Authorities

The Sponsor or a Contract Research Organization (CRO) will be responsible for reporting all UADEs to FDA. Principal Investigator and IRBs, as applicable, in accordance with national regulations in the country where the study is conducted.

Relative to the first awareness of the event by/or further provision to the Sponsor or Sponsor's designee, UADEs will be submitted to IRB and FDA within 10 days.

Institution and/or Principal Investigator shall promptly, in accordance with applicable laws, rules and regulations and the procedures specified in the Protocol, advise Sponsor and the IRB, as applicable of any adverse events and adverse reactions occurring during the conduct of the Study that become known to it/him/her. Without limiting the generality of the foregoing, Institution and/or Principal Investigator shall notify Sponsor or the medical monitor of any serious adverse events and serious adverse reactions encountered in the Study (including, without limitation, all serious and unexpected adverse reactions that the Principal Investigator suspects may be Study Device related) via email within 24 hours of making such discovery to enable Sponsor to timely report such events in accordance with applicable laws, rules and regulations as required to and any applicable regulatory authority including, as applicable the FDA. Additionally, Institution and/or Principal Investigator shall: (i) provide all communications with the IRB to Sponsor; and (ii) cooperate with Sponsor to investigate the Adverse Event and provide all documentation requested by Sponsor.

11.0 DATA HANDLING AND MANAGEMENT

Clinical Data Management (CDM) is the responsibility of ProSciento, Chula Vista, USA. With permission of the Sponsor, Data Management may be delegated under an agreement of transfer of responsibilities to a qualified vendor of ProSciento.

11.1 Data Management

The full details of procedures for data handling will be documented in the Data Management Plan (DMP).

ADEs and medical history will be coded using the current version of the Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medications will be coded using the World Health Organization (WHO) WHODrug Global dictionary. The MedDRA version used will be recorded in the Study Master File documentation.

Unique numbers will identify the subject and the biological material obtained from the subject. Appropriate measures such as encryption or deletion will be enforced to protect the identity of human subjects in all presentations and publications as required by local/regional/national requirements, and data exchanges with sponsor.

Data from screening failures will be entered into the database. The data for screen failures will be limited to ICF date, demographics, and eligibility assessment including reason(s) for screen fail.

Laboratory data from the central laboratory will be electronically transferred to ProSciento for database reconciliation purposes and loading into EDC for data review purposes.

The laboratory will provide one copy of the laboratory reports to the site staff. The site staff will receive all laboratory data electronically or based on FAX reports directly from the laboratory. The Principal Investigator must review, evaluate, sign and date the laboratory printouts upon receipt. The signed print-out of the laboratory reports are source data.

All other results and laboratory tests will be transferred electronically to the responsible Data Management Unit.

Clamp and CGMS data will be prepared for data transfer by ProSciento, and provided to the Sponsor (or through designated CROs).

11.2 CRFs (Electronic)

11.2.1 Clinical Data Management Workflow

Electronic CRFs will be developed by ProSciento's Clinical Data Management (CDM) or an authorized CDM department, in collaboration with the clinical study team and statistician. CDM will document the process workflow in the DMP. After data entry, monitor(s) will source data verify (SDV) the eCRFs against the source documents. Queries may be issued to clarify the data entered. The PI will electronically sign the

eCRFs after all data have been entered and all queries have been resolved. If corrections and/or resolution of queries are required after PI approvals, those eCRFs affected by changes will be re-signed by the PI. The database may be locked after the PI approvals are completed.

After database lock, CDM study design documentation and locked eCRFs (PDF) will be created and will be provided to the Sponsor.

11.2.2 Data Entry of eCRFs

Data required for analyses and subject safety assessments will be entered from source documentation into eCRFs. Instructions for data entry will be provided in the eCRF Completion Guidelines, developed by ProSciento's CDM department. All site staff involved with entering data into the eCRFs will be trained prior to gaining access to the study database.

11.2.3 Corrections to eCRFs

Queries may be generated by the eCRF system during data entry, and queries may be generated by CDM staff, monitors, PIs, the Sponsor, and other data reviewers during the course of the study. Only specific site personnel will be authorized to make corrections to the eCRFs; CDM will train personnel prior to granting access in the eCRF system. Corrections will be made directly in the eCRF – by modifying existing data, adding new data, or deleting data, as appropriate. All data corrections will be logged in the electronic audit trail.

11.2.4 PI Approval of eCRF Data

The Principal Investigator or authorized staff must ensure that all information derived from source documentation is consistent with the source information and accurately reflected in the eCRFs. By electronically signing the eCRFs, the Principal Investigator confirms that the information is complete and correct.

11.3 Retention of Documents

At the completion of the study, all records created by and under the supervision of the Principal Investigator should be maintained in accordance with the requirements of the regulatory authority guideline and the GCP Guideline. These will be available for inspection at any time by the Sponsor or the FDA.

Clinical study documents are archived upon completion of the study and maintained for at least 15 years from the study closure or longer in accordance with local regulation and applicable regulatory authority guidelines, and the study sponsor will be notified prior to destruction of study records.

12.0 STATISTICAL METHODS

12.1 Statistical and Analytical Plans

ProSciento or qualified designee will be responsible for the statistical services, including statistical analysis, statistical reports and the statistical analysis plan (SAP).

An SAP will be prepared prior to database lock, that will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives. It will also provide any changes or additions to the analyses that are not apparent in the protocol.

12.1.1 Analysis Sets

12.1.1.1 Safety Set

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.

12.1.1.2 Ultrasound Insonification Effect Set

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.

12.1.2 Analysis of Demographics and Other Baseline Characteristics

Demographic and baseline characteristics will be summarized for all subjects. Summary statistics (e.g., number of subjects, mean, median, standard deviation, and range) 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.

12.1.3 Analysis of the Endpoints for Ultrasound Insonification

12.1.3.1 Two-Step Hyperinsulinemic-Euglycemic Clamp Analysis

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.

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

Insulin Sensitivity Index (SI_{clamp})

The insulin sensitivity index (SI_{clamp}) will be calculated 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).

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

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.

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.

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

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

Insulin ratio (M/I) will be calculated for each clamp and ratios will be compared. 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 planned analyses will be described in more detail in the statistical analysis plan (SAP).

12.1.3.2 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, plasma glucose enrichments will be measured to determine steady state and to determine fasting EGP using steady-state equations (Steele 1959).

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.

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-$^2H_2$]glucose during constant infusion of [6,6-$^2H_2$]glucose. Rd will be calculated during steady state in the fasting period and during Step 1 and Step 2 of the clamp.

12.1.4 Safety Analysis and Endpoints

Safety of the procedure will be assessed by collection and review of adverse device effects, laboratory parameters, physical examination, vital signs, and ECG parameters throughout the duration of the study. Safety analysis will involve examination of the descriptive statistics and individual subject listings for effects of study treatment on clinical tolerability and safety.

Safety data will be summarized using the safety analysis set.

12.1.4.1 Adverse Device Effects

All ADEs will be coded using MedDRA. Data will be summarized using preferred term and primary system organ class.

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 adverse events.

ADE data and UADEs will be listed by subject.

12.1.4.2 Vital Signs, ECGs and Physical Examinations

Absolute values and changes from baseline will be assessed for vital sign and ECG parameters at specified timepoints. Abnormal findings will be listed. Graphical presentation of vital sign and ECG parameters at specified timepoints may be provided.

Changes in physical examination at each timepoint will be listed by subject.

12.1.4.3 Clinical Laboratory Assessments

Absolute values and changes from baseline will be assessed for hematology, serum chemistry and urinalysis parameters. Post-baseline shifts in parameter values will be presented for the worst on-study observed values. Clinically significant laboratory parameters will be listed.

All laboratory data will be listed and will include a flag for abnormal high or low values and clinically significant results.

12.2 Interim Analysis

There are no planned interim analyses.

12.3 Determination of Sample Size

No formal sample size calculations were performed as this is an exploratory study.

13.0 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Monitoring

The study will be monitored by M Squared Associates Inc., 575 Eighth Avenue, Suite 1212, New York, NY 10018.

Monitoring visits to the study site will be made periodically during the study to ensure that all aspects of the protocol and GCP are followed, CRFs are completed correctly, and subjects' safety is monitored. The Monitor will conduct a detailed training before First Subject First Visit (FSFV) (Initiation Visit), a monitoring visit at least once during the clinical part of the study, and a monitoring visit at least once after Last Subject Last Visit (LSLV). Furthermore, the Monitor will be available and maintain direct communication with the study site by telephone and e-mail throughout the trial.

To ensure investigator compliance per 21 CFR 812.46, monitoring of the investigational sites will be conducted by the sponsor or the sponsor's representative according to the monitoring plan developed specifically for this study. To ensure that the investigator and site staff understand and accept the defined responsibilities, the monitor will maintain regular correspondence with the site during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant FDA regulations, and the maintenance of complete records. Monitoring will include review and resolution of missing or inconsistent results and source document checks (i.e., comparison of submitted study results to original reports) to assure the accuracy of the reported data.

The investigators and institutions will permit the sponsor or sponsor's representative to perform study-related monitoring, audits, IRB review, and regulatory inspections by providing direct access to source data/documentation.

Clinical Research Associates (CRAs) of ProSciento, Inc., 855 3rd Avenue, Chula Vista, CA 91911, will monitor the clamp data of this study.

The Monitor must be given direct access to source documents, such as original documents, data, and records. Direct access includes permission to examine, analyze, verify any record(s) and report(s) that are important to evaluation of the clinical study. The study will be monitored to verify integrity and validity of the data. Monitoring will follow a Monitoring Plan.

Additional QC monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by qualified staff of ProSciento, Inc.

13.2 Protocol Deviations

The Principal Investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study subjects. Should other circumstances arise that will require deviation from protocol-specified procedures, unless there is an emergency or immediate need, the Principal Investigator should contact the medical monitor and

Sponsor to review and discuss the implications of the deviation and determine the appropriate course of action. Any deviation must be documented, stating the reason and date, the action taken, and the impact for the subject and/or the study. The documentation must be kept in the Principal Investigator's Study File and the Sponsor's Study Master File.

14.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted in accordance with the Protocol, the International Conference on Harmonization (ICH), Guideline for Good Clinical Practice: Consolidated Guidance (E6 R2) and applicable regulatory requirements including clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 11, 50, 54 and 56, and is subject to the abbreviated requirements of the investigational device exemptions (IDE) defined in the US 21 CFR 812.2(b), and the principles enunciated in the Declaration of Helsinki (revised version Fortaleza 2013).

14.1 Institutional Review Board

Prior to commencement of the study, the protocol, any amendments, subject information/informed consent form, any other written information to be provided to the subject, subject recruitment procedures, information about payments and compensation available to subjects if not mentioned in the subject information, the Principal Investigator's current CV and/or other documentation evidencing qualifications, and other documents as required by the local Institutional Review Board (IRB) should be submitted. Written approval/favorable opinion must be obtained from IRB prior to commencement of the clinical study start.

The Principal Investigator shall provide to Sponsor or its designee a copy of the written and dated approval/favorable opinion by the applicable the IRB.

During the study, the Principal Investigator must promptly report the following to the IRB: UADEs, substantial amendments to the protocol, non-substantial amendments, deviations to the protocol implemented to eliminate immediate hazards to the subjects, new information that may affect adversely the safety of the subjects or the conduct of the study (including new risk/benefit analysis in case it will have an impact on the planned follow-up of the subjects), annually written summaries of the study status and other documents as required by the local IRB.

Substantial amendments must not be implemented before approval/favorable opinion, unless necessary to eliminate hazards to the subjects.

The Principal Investigator must maintain an accurate and complete record of all submissions made to the IRB. The records should be filed in the Principal Investigator's Study File and copies must be provided to the Sponsor.

14.2 Responsibilities of the Principal Investigator

The Principal Investigator will conduct this clinical study in compliance with all applicable national, state, local or regional laws. The Principal Investigator will align his or her conduct in accordance with the "Responsibilities of the Principal Investigator".

The Principal Investigator may appoint other individuals as he/she may deem appropriate as Sub-Investigators to assist in the conduct of the Clinical Study in accordance with the Clinical Study Protocol. All Sub-Investigators shall be timely appointed and listed. The

Sub-Investigators will be supervised by and under the responsibility of the Principal Investigator. The Principal Investigator will provide them with a Clinical Study Protocol and all necessary information and document training and adhere to all Investigator Requirements (e.g., providing financial disclosure, curriculum vitae [CV], signed investigator agreement, etc.). Each Sub-Investigator is responsible for fulfilling all of the obligations directed to them by the Principal Investigator.

In compliance with the Clinical Study Protocol, Good Clinical Practice and applicable regulatory requirements, the Principal Investigator must permit auditing by or on the behalf of the Sponsor and inspection by applicable regulatory authorities. The Principal Investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that these personnel are bound by professional secrecy and as such will not disclose any personal identity or personal medical information. The Principal Investigator will make every effort to help with the performance of the audits and inspections, giving access to all necessary facilities, data, and documents. As soon as the Principal Investigator is notified of a future inspection by the authorities, he or she will inform the Sponsor or its designee and authorize the Sponsor or its designee to participate in this inspection. Any results and information arising from the inspections by the regulatory authorities will be immediately communicated to the Sponsor. The Principal Investigator shall take appropriate measures required by the Sponsor to take corrective actions for all problems found during the audit or inspections.

Clinical research studies sponsored by the Sponsor are subject to ICH GCP and all the applicable local laws and regulations.

14.3 Informed Consent

Once signed, the original informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) will be stored in the Principal Investigator's site file. The Principal Investigator must document the date the subject signs the informed consent in the subject's medical record. Copies of the signed informed consent form, the signed subject authorization form (if applicable), and subject information sheet (if applicable) shall be given to the subject.

All revised informed consent forms must be reviewed and signed by relevant subjects or the relevant subject's legally acceptable representative in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the subject's medical record, and the subject should receive a copy of the revised informed consent form.

14.4 Subject Confidentiality

The Sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Subject records will be kept private except when ordered by law. The following individuals will have access to study subject records:

Principal Investigator and designees, study Sponsor, monitors and auditors, the FDA, other government offices and the IRB.

Throughout this study, a subject's source data will only be linked to the Sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

The Principal Investigator must agree to permit the Sponsor's monitor or designee's monitor, representatives from any regulatory authority, the Sponsor's designated auditors, and the appropriate IRB to review the subject's source data or documents, including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process. The confidentiality of the verified data and the protection of the subjects must be respected during these inspections.

Copies of any subject source documents that are provided to the Sponsor must have certain personally identifiable information removed.

14.5 Property Rights

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf), or produced during the Clinical Study, including but not limited to, the Clinical Study Protocol, the case report forms, the Principal Investigator's Brochure and results obtained during the course of the Clinical Study, is confidential and the property of the Sponsor. No rights, title, or license to such data are granted to ProSciento by this protocol. The Principal Investigator and all persons under his/her authority agrees to undertake to keep confidential and not to disclose the information to any third party without the prior written approval of the Sponsor.

All information, documents and study drug provided by Sponsor or its designee are and remain the sole property of the Sponsor. The Principal Investigator shall not mention any information or the study drug in any application for a patent or for any other intellectual property rights.

All results, data, documents and inventions, which arise directly or indirectly from the Clinical Study in any form, shall be the immediate and exclusive property of the Sponsor. The Sponsor may use or exploit all results at its own discretion, without limitation to its property right (territory, field, continuance). The Sponsor shall be under no obligation to patent, develop, market or otherwise use the results of the Clinical Study.

14.6 Publication, Disclosure, and Clinical Study Registration Policy

The Principal Investigator will provide the Sponsor with truthful, accurate and complete test results and all data derived from the study. During the study, only the Sponsor may

make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility and prerogative of the Sponsor.

ProSciento or its designee and Sponsor will be responsible for preparing the Clinical Study Report. When all data has been fully analyzed, ProSciento or Sponsor will communicate the results of the Clinical Study to the Principal Investigator(s) at Sponsor's sole option.

The Principal Investigator or qualified designee agrees to use this information only and strictly in connection with this Clinical Study and not for other purposes without the prior written permission from the Sponsor. ProSciento and the Principal Investigator may publish or present the results of the Clinical Study or any data derived from the Study only in accordance with the requirement of Section 9.1 of the Clinical Study Agreement having an effective date of 19 December 2019. Prior to any publication, the Sponsor must be given the opportunity to review and comment upon any manuscript, poster, or paper that contains data derived or generated from this study in order to be aware of all written and oral presentations of the data.

The study may be registered on publicly accessible websites (e.g., ClinicalTrials.gov) according to applicable law, regulation and guidance, after discussion with the Sponsor.

15.0 REFERENCES

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16.0 APPENDIX

Table 16-1 Schedule of Events (SOE)

ASSESSMENTS	SCREEN	IN-HOUSE PERIOD 1			OPV	IN-HOUSE PERIOD 2				OPV	F/U
		Observational Period 1 (Days -1 to Day 14)				Insonification (Days 15-17)			Observational Period 2 (Days 18 to Day 28)		
Visit/ Timepoint (Day)	Start Day -28	-1	1	2	8	15	16	17	18	22	28
Study Intervention/ Insonification						X	X	X			
Informed consent	X										
Assess eligibility criteria	X										
Demography	X										
Medical history	X										
Sequestered in clinic/unit		X	X	X		X	X	X	X		
Physical examination (PE)	X										
Abbreviated PE		X				X			X		X
Hematology, serum chemistry & urinalysis	X	X				X			X		X
Coagulation	X										
TSH	X										
HbA1c	X	X				X			X		X
Hep B, Hep C, HIV	X										
Pregnancy test (urine)		X			X	X				X	X
Pregnancy test (serum)	X										
FSH if postmenopausal	X										
Urine drug screen & alcohol breath test	X	X			X	X				X	X
Weight, BMI	X	X	X		X	X		X		X	X
Height	X										
Vital signs	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X		X		X		X			X
Instructions on SMBG /glucose via fingerstick to measure during outpatient periods (Days 3-14 and 19-27)		X									

ASSESSMENTS	SCREEN	IN-HOUSE PERIOD 1			OPV	IN-HOUSE PERIOD 2				OPV	F/U
		Observational Period 1 (Days -1 to Day 14)				Insonification (Days 15-17)			Observational Period 2 (Days 18 to Day 28)		
Visit/ Timepoint (Day)	**Start Day -28**	**-1**	**1**	**2**	**8**	**15**	**16**	**17**	**18**	**22**	**28**
Study Intervention/ Insonification						**X**	**X**	**X**			
Record concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Record ADEs	X	X	X	X	X	X	X	X	X	X	X
Standardized meals		X	X	X		X	X	X	X		
CGMS		X	X	X	X	X	X	X	X	X	X
CGMS sensor insertion/ change		X			X				X		
Overnight fast ≥ 10 hours			X	X	X	X	X	X	X	X	X
Check of imaging criteria						X					
OGTT			X				X				
Insulin and glucose tracer infusion overnight			X	X			X	X			
Blood sampling for EGP				X				X			
Initiation of Clamp procedure				X				X			
Fasting plasma glucose			X	X	X	X	X	X	X	X	X
HOMA-IR			X			X			X		X
Exploratoory biomarkers for assessment and potential storage			X			X			X		X
Glucose parameters (e.g., Fructosamine)			X			X			X		X
Lipid meatabolism parameters (e.g. FFA, triglycerides)			X			X			X		X
Inflammatory biomarkers (e.g., adipnectin, CRP)			X			X			X		X

Table 16-2 Schedule of Events (SOE) for Subjects with BMI> 35.0 kg/m^2 and ≤ 40.0 kg/m^2 and/or Waist Circumference > 40 and ≤ 45 inches

ASSESSMENTS	SCREENI PERIOD	IN-HOUSE PERIOD 1			OPV	IN-HOUSE PERIOD 2				OPV	F/U
		Observational Period 1 (Days -1 to Day 14)				Insonification (Days 15-17)			Observational Period 2 (Days 18 to Day 28)		
Visit/ Timepoint (Day)	Start Day—50	-1	1	2	8	15	16	17	18	22	28
Study Intervention/ Insonification						**X**	**X**	**X**			
Informed consent	X										
Assess eligibility criteria	X										
Re-assess eligibility criteria		X**									
Demography	X										
Medical history	X										
Sequestered in clinic/unit		X	X	X		X	X	X	X		
Physical examination (PE)	X										
Abbreviated PE		X				X			X		X
Hematology, serum chemistry & urinalysis	X	X				X			X		X
Coagulation	X										
TSH	X										
HbA1c	X	X				X			X		X
Hep B, Hep C, HIV	X										
Pregnancy test (urine)		X			X	X				X	X
Pregnancy test (serum)	X										
FSH if postmenopausal	X										
Urine drug screen & alcohol breath test	X	X			X	X				X	X
Weight, BMI	X	X	X		X	X		X		X	X
Height	X										
Waist circumference	**X**										
Hepatic ultrasound (Screening only)	**X**										
Vital signs	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X		X		X		X			X

ASSESSMENTS	SCREENI PERIOD	IN-HOUSE PERIOD 1			OPV	IN-HOUSE PERIOD 2				OPV	F/U
		Observational Period 1 (Days -1 to Day 14)				Insonification (Days 15-17)			Observational Period 2 (Days 18 to Day 28)		
Visit/ Timepoint (Day)	Start Day—50	-1	1	2	8	15	16	17	18	22	28
Study Intervention/ Insonification						X	X	X			
Instructions on SMBG /glucose via fingerstick to measure during outpatient periods (Days 3-14 and 19-27)		X									
Record concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Record ADEs	X	X	X	X	X	X	X	X	X	X	X
Standardized meals		X	X	X		X	X	X	X		
CGMS		X	X	X	X	X	X	X	X	X	X
CGMS sensor insertion/ change		X			X				X		
Overnight fast ≥ 10 hours			X	X	X	X	X	X	X	X	X
Check of imaging criteria						X					
OGTT			X				X				
Insulin and glucose tracer infusion overnight			X	X			X	X			
Blood sampling for EGP				X				X			
Initiation of Clamp procedure				X				X			
Fasting plasma glucose			X	X	X	X	X	X	X	X	X
HOMA-IR			X			X			X		X
Exploratoory biomarkers for assessment and potential storage			X			X			X		X
Glucose parameters (e.g., Fructosamine)			X			X			X		X
Lipid meatabolism parameters (e.g. FFA, triglycerides)			X			X			X		X
Inflammatory biomarkers (e.g., adipnectin, CRP)			X			X			X		X

*Study Visit to be performed ≥ 30 days after hepatic ultrasound screening examination.
**Necessary laboratory analyses and examinations need to be performed prior to Day -1, so that all eligibility criteria from screening can be re-assessed on Day -1. Re-assessment can be performed after a 30-day wait period, starting after the ultrasound examination.

Table 16-3 Toronto Clinical Neuropathy Scoring System

Toronto Clinical Neuropathy Scoring System
Three aspects comprise the test – a patient questionnaire, a simple sensory test (refer to methods on the following page), and a reflex test. Any score > 8 on the test is considered moderate or severe peripheral neuropathy, and those patients will be excluded. An additive score of 8 or less is considered mild or non-existent peripheral neuropathy, and those patients will be permitted to participate in the study.

Symptom Scores	Left 0 = Absent 1= Present	Right 0 = Absent 1= Present
Foot Pain		
Numbness		
Tingling		
Weakness		
Ataxia		
Upper Limb Symptoms		
Total symptom Score		

Sensory Scores	Left 0 = Normal 1= Abnormal	Right 0 = Normal 1= Abnormal
Pinprick *(Neurotip)*		
Temperature *(5.07/ 10-g monofilament)*		
Light Touch		
Vibration *(128-Hz tuning fork)*		
Position Sense		
Total Sensory Score		

Reflex Scores	Left 0 = Normal 1= Reduced 2 = Absent	Right 0 = Normal 1= Reduced 2= Absent
Knee Reflex		
Ankle Reflex		
Total Reflex Score		

Total Additive Score		
Component	**Left**	**Right**
Questionnaire		
Sensory		
Reflex		
Total		
	INV INITIALS	