AdventHealth Orlando
Translational Research Institute for Metabolism and Diabetes

STUDY PROTOCOL TITLE

Evaluation of Biomarkers to Quantify Liver Pathology in Patients with Presumed Non-Alcoholic Steatohepatitis at Baseline and Following Bariatric Surgery

STUDY SPONSOR

AdventHealth Orlando

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACE	angiotensin converting enzyme
AE	adverse event
ALT	alanine aminotransferase
AMRA	Advanced MR Analytics AB
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
BMI	body mass index
CAP	College of American Pathologists
CAP™	Controlled Attenuation Parameter
CBC	complete blood count
CJD	Creutzfeldt-Jakob disease
CMP	comprehensive metabolic panel
CSR	clinical study report
DNA	deoxyribonucleic acid
DSI	Disease Severity Index derived from HepQuant SHUNT Dual Cholate Liver Diagnostic Kit
DUA	Data Use Agreement
d4-cholate	Cholic Acid-2,2,4,4-d4
eCRF	electronic case report form
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA1C	hemoglobin A1C
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HCVAb	hepatitis C virus antibody
HIV	human immunodeficiency virus
HSA	human serum albumin
ICH	International Council on Harmonisation
IHL	intrahepatic lipid
IND	Investigational New Drug
INR	international normalized ratio
IRB	institutional review board
IV	intravenous
LC-MS	liquid chromatography-mass spectrometry

Abbreviation	Definition
LMS	LiverMultiScan
LSM	Liver Stiffness Measurement
MAR	missing at random
MELD	model of end-stage liver disease
MI	multiple imputation
MR	magnetic resonance
MRE	magnetic resonance elastography
MRI	magnetic resonance imaging and spectroscopy
MRI-PDFF	magnetic resonance imaging proton density fat fraction
MRS-IHL	magnetic resonance spectroscopy intrahepatic lipid
MTA	Material Transfer Agreement
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
PBS	phosphate buffered saline
PHI	personal health information
PID	participant identification number
PK	pharmacokinetic
PT	prothrombin time
PTH	parathyroid hormone
PTT	partial thromboplastin time
RNA	ribonucleic acid
RNA-Seq	RNA sequencing
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
SOP	standard operating procedure
SSP	study specific procedure
TRI-MD	Translational Research Institute for Metabolism and Diabetes
TSH	thyroid stimulating hormone
T4	thyroxine
US	United States
vCJD	variant Creutzfeldt-Jakob disease
VCTE™	Vibration-Controlled Transient Elastography
WI	work instruction
13C-cholate	Cholic Acid-24-13C
β-hCG	beta subunit human chorionic gonadotropin

INTRODUCTION

This document is a protocol for a human research study to evaluate imaging, circulating and microbiome biomarkers of liver pathology in patients with non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH). The described study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) International Council on Harmonisation (ICH) Guidelines (E6) for GCP standards as adopted by the Food and Drug Administration (FDA) and associated Federal regulations, and all applicable institutional research requirements.

BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

The rise in prevalence of obesity across the United States (US) is accompanied by a host of cardiometabolic comorbidities, including NAFLD, which is now the most common cause of chronic liver disease in industrialized nations (Ahmed 2010, Loomba 2013). Indeed, NAFLD affects approximately 20–30% of normal weight individuals and 57–98% of obese individuals depending on population and diagnostic modality (Vernon 2011). Within the spectrum of NAFLD phenotypes, NASH is a condition that is distinguished from simple steatosis in that it includes necroinflammatory changes in addition to steatosis. NASH is a burgeoning liver disease (estimated prevalence is 3–5% of the US population) for which no approved therapeutic treatment currently exists (Sanyal 2015).

Approximately 25% of those diagnosed with NAFLD meet the histopathologic criteria for NASH (Cohen 2011), which unlike the clinically benign condition of simple steatosis, poses a higher risk of progression to cirrhosis and development of hepatocellular carcinoma (HCC). A major gap in the field is the inability to identify those who are at risk for progression from benign NAFLD to severe stages of NASH such as fibrosis, cirrhosis, and HCC.

With treatment of NAFLD/NASH being a major public health objective, several promising therapies have recently entered advanced clinical trials. However, one of the primary challenges hindering development of treatments for NAFLD/NASH is the lack of non-invasive tools to accurately diagnose and stage the disease. Currently, most clinical trials examining chronic liver disease rely heavily on histologic evaluation of a liver biopsy sample. This diagnostic assay is associated with a number of drawbacks. In addition to risk of complications associated with the invasive nature of the biopsy procedure, the limited volume of tissue collected by the biopsy introduces sampling error, while interpretation and scoring of the histology itself is a somewhat subjective process that can introduce variability to the assessment (Raitziu 2005). Therefore, there is a recognized need within the field to develop a non-invasive alternative to the liver biopsy for diagnosing and staging NAFLD/NASH. Such a development would enable the design of clinical trials to assess efficacy of prospective compounds to treat NASH without the need for liver biopsies, thereby improving safety 17and facilitating recruitment, which, in turn, would speed up the translation of new drugs to patients who are in need of treatment options.

Several methods have been studied for non-invasive diagnosis of NAFLD and NASH, however none have been found to sufficiently correlate with stages of disease progression or NASH diagnosis to substitute for liver biopsy in either the clinical care of patients or selection of patients for clinical trials (Alkhouri 2012, Papagianni 2015, Renelus 2016). Among these methodologies, medical imaging approaches have garnered significant interest within the

NAFLD research community given their potential to directly probe the extent of different pathophysiologic mechanisms within the liver itself (Dulai 2016, Hashemi 2016).

Over the course of this study, longitudinal assessments of subjects with NASH will be performed using non-invasive imaging (FibroScan® and magnetic resonance imaging [MRI] methods) to evaluate the effects of bariatric surgery on the liver and on extra hepatic depots of adipose tissue. A battery of circulating, functional, and microbiome-based assays will also be employed to evaluate physiologic changes that occur in response to bariatric surgery (OWLiver Care analysis to discriminate between NAFLD and healthy liver; OWLiver Care analysis to discriminate between NASH and simple steatosis; Nordic Bioscience panel to interrogate fibrogenic activity; HepQuant SHUNT Dual Cholate Liver Diagnostic Kit test to monitor the effect of bariatric surgery on liver function; and stool sample assays of the gut microbiome to assess the effect of bariatric surgery on the composition, functionality, and metabolites of the microbiota) (see the Research Design section for detailed information and background on these procedures and assessments).

A population of patients undergoing standard of care bariatric surgery has been chosen because of the known effect of bariatric surgery on NASH. Roux-en-Y gastric bypass is associated with decreased hepatic steatosis (with total resolution in 55.5% of patients) and resolution of hepatic inflammation and ballooning degeneration of hepatocytes (100.0 and 88.8% of patients, respectively), with histologic improvement including decreases in lobular inflammation and portal/lobular fibrosis (Barker 2006, Rabl 2012, Hafeez 2013, Schneck 2016), all in addition to functional liver recovery (Alizai 2015).

STUDY OBJECTIVES

The table below lists each objective along with its corresponding endpoint.

Table 1: Study Objectives and Endpoints

Co-Primary Objectives and Endpoints	
Objective	**Endpoint**
To evaluate the effect of bariatric surgery on liver fat in adults with non-alcoholic steatohepatitis (NASH) 84 days (12 weeks) post-surgery	Percent change from baseline in liver fat at 84 days (12 weeks) post-surgery, as assessed by magnetic resonance imaging proton density fat fraction (MRI-PDFF)
To evaluate the effect of bariatric surgery on liver stiffness in adults with NASH 84 days (12 weeks) post-surgery	Percent change from baseline in liver stiffness at 84 days (12 weeks) post-surgery, as assessed by magnetic resonance elastography (MRE)
Secondary Objectives and Endpoints	
Objectives	**Endpoint**
To evaluate the effect of bariatric surgery on liver fat and liver stiffness in adults with NASH 84 days (12 weeks) post-surgery	Percent change from baseline in liver fat and liver stiffness at 84 days (12 weeks) post-surgery, as assessed by FibroScan®-derived Controlled Attenuation Parameter (CAP™) and stiffness

Secondary Objectives and Endpoints	
Objectives	**Endpoint**
To evaluate the effect of bariatric surgery on liver fat and liver stiffness in adults with NASH over time post-surgery	Percent change from baseline in liver fat and liver stiffness over time post-surgery (42 days [6 weeks], 84 days [12 weeks], 182days [26 weeks], and 364 days [52 weeks]), as assessed by: • FibroScan®-derived CAP™ and stiffness • MRI-PDFF, magnetic resonance spectroscopy intrahepatic lipid (MRS-IHL), and MRE
To evaluate the effect of pre-surgical dietary intervention on liver fat and liver stiffness in adults with non-alcoholic fatty liver disease (NAFLD) (entire study population)	Percent change in liver fat and liver stiffness between screening and baseline, as assessed by: • FibroScan®-derived CAP™ and stiffness • MRI-PDFF and MRE
Exploratory Objectives and Endpoints	
Objective	**Endpoint**
To evaluate the effect of bariatric surgery on extrahepatic adipose depots, skeletal muscle, and liver fibro-inflammatory state in adults with NASH over time	Percent change from baseline, over time post-surgery (42 days [6 weeks], 84 days [12 weeks], 182 days [26 weeks], and 364 days [52 weeks]), in: • extrahepatic volumes of adipose tissue and skeletal muscle as assessed by MRI body composition profiling • corrected-T1 of the liver as assessed by LiverMultiScan MRI
To evaluate the effect of bariatric surgery on kinetic measures of liver function in adults with NASH over time	Percent change from baseline, over time post-surgery (42 days [6 weeks], 84 days [12 weeks], 182 days [26 weeks], and 364 days [52 weeks]), in Disease Severity Index (DSI) and %-shunt as assessed by HepQuant SHUNT
To evaluate the effect of bariatric surgery on gut microbiome in adults with NASH over time	Change from baseline, over time post-surgery (42 days [6 weeks], 84 days [12 weeks],182 days [26 weeks], and 364 days [52 weeks]), in stool sample: • composition (shotgun analysis of microbiome DNA) • functionality (shotgun analysis of microbiome RNA) • metabolites of microbiota
To evaluate the effect of bariatric surgery on circulating metabolomics, protein biomarkers, and liver enzymes in adults with NASH over time	Percent change from baseline, over time post-surgery (42 days [6 weeks], 84 days [12 weeks], 182 days [26 weeks], and 364 days [52 weeks]), in: • OWLiver, and OWLiver Care metabolomics panels from OWL • pro-collagen protein fragments panel from Nordic Bioscience • aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes
To evaluate pre-surgery differences in biomarker signatures between adults with NASH and those with non-NASH NAFLD	Difference measures between subject groups at baseline for: • FibroScan®-derived CAP™ and stiffness • magnetic resonance (MR)-derived measures • circulating biomarker measures • gut microbiome measures
To assess predictive nature of measured biomarkers to non-invasively characterize disease state in adults with NAFLD (entire study population)	Correlative relationship between disease pathobiology as assessed from histopathology measures of biopsy samples (liver) collected during bariatric surgery and any other secondary or exploratory endpoints

Exploratory Objectives and Endpoints	
Objective	**Endpoint**
To evaluate the effect of bariatric surgery on liver fat in adults with non-alcoholic fatty liver disease (NAFLD) (entire study population)	Percent change in intrahepatic lipids by magnetic resonance spectroscopy (MRS-IHL)
To evaluate the ability of a diffusion-based MRI method to non-invasively measure liver stiffness in adults with NAFLD (entire study population)	Correlation between liver stiffness measures derived from diffusion-based MRI and those methods that impart a mechanical perturbation to tissue, namely: • FibroScan®-derived stiffness • MRE

STUDY DESIGN

Research Design

This is a non-randomized, single-site, biomarker methodology study in subjects with presumed NAFLD and NASH undergoing bariatric surgery. Participants will undergo bariatric surgery along with all pre- and post-surgery medical visits and standard of care laboratory assessments.

Over the course of this study, longitudinal assessments of subjects with NASH or non-NASH NAFLD will be performed using a battery of non-invasive circulating, functional, imaging, and microbiome-based assays to evaluate physiologic changes that occur in response to bariatric surgery.

The specific imaging to be employed in this study includes FibroScan® and MRI to noninvasively evaluate the effects of bariatric surgery on the liver and on extra-hepatic depots of adipose tissue. The FibroScan® instrument applies a technique referred to as vibration-controlled transient elastography (VCTE™) to simultaneously derive measures of stiffness for the assessment of liver fibrosis, and of ultrasound wave attenuation for the assessment of liver steatosis (a measure referred to as the controlled attenuation parameter [CAP™]). Magnetic resonance (MR) assessments will apply scanning methods to interrogate steatosis, inflammation, and fibrosis of the liver through measures derived from proton density fat fraction (MRI-PDFF), proton spectroscopy (MRS-IHL), T1 relaxometry using the LiverMultiScan (LMS) protocol, and MR elastography (MRE), respectively. To assess effects of surgical intervention outside the liver, MRI evaluation of extra-hepatic adipose tissue volumes within visceral and subcutaneous regions, and skeletal muscle will be quantified.

This study will also collect blood samples to investigate the effect of bariatric surgery on longitudinal profiles of several categories of circulating biomarkers and evaluate their utility in tracking NASH disease regression.

Recent advances in metabolomic testing methodologies and associated analysis algorithms have led OWL Metabolomics to define lipidomic signatures for different NAFLD subtypes, and for differentiating NASH from simple steatosis (Barr 2012). Analysis of serum lipid metabolites by two separate logistic regression algorithms was conducted in a recent study of biopsy-proven NAFLD patients. Findings from that study demonstrated that the OWLiver Care analysis is able to discriminate between NAFLD

and healthy liver, while the OWLiver analysis is able to discriminate between NASH and simple steatosis, both with AUROC >0.9 (Crespo 2016). Similarly, assay development to quantify circulating proteins associated with tissue remodeling has led Nordic Bioscience to identify a panel to interrogate fibrogenic activity (Nielsen 2013). In a recent clinical study of patients with advanced hepatitis C, profiling markers related to both synthesis and degradation of the extracellular matrix (in the form of collagen pro-peptides and collagen degradation fragments) was found to serve a prognostic role by enabling identification of patients likely to respond to anti-fibrotic therapy (Nielsen 2015). As similar fibrogenic processes are likely active in NAFLD patients that are at risk of advancing to NASH, there is interest to determine if the panel may provide similar prognostic value, and if assessed longitudinally, insight to therapeutic efficacy in advance of histological or clinical assessments.

This study will also monitor the effect of bariatric surgery on liver function as characterized by the HepQuant SHUNT Dual Cholate Liver Diagnostic Kit test. This diagnostic test is being developed as a quantitative assay of liver function that involves the co-administration of stable isotopes of cholate: 13C-cholate [cholic acid-24-13C, IND #65,121] (administered intravenously [IV]); and 4d-cholate [cholic acid-2,2,4,4-d4, IND #65,123] (administered orally). Following the dual-cholate administration, timed blood samples are collected over a 90-minute period. Liquid chromatography-mass spectrometry (LC-MS) analysis of the samples will generate kinetic measures of cholate clearance. Clearances of IV- and orally-administered cholate are liver-specific functions dependent upon systemic and portal blood flows, respectively, and hepatocyte uptake. The primary output of the test is a summary parameter referred to as the Disease Severity Index (DSI), which is calculated from the clearance rates. DSI values increase with impairment of cholate clearances and have been shown to correlate with increasing severity of liver disease and risk for adverse clinical outcomes (Helmke 2015). A standard hepatic panel used clinically to monitor liver health will also be collected.

The effect of bariatric surgery on gut microbiome will be assessed through stool sample assays for composition, functionality, and metabolites of microbiota (both stool and circulating). Alterations to the intestinal microbiota have been found to contribute to the pathogenesis of many liver diseases, including NAFLD (Schnabl 2014). Though the mechanism through which microbiota may negatively or positively influence the onset of NAFLD is not fully understood, it is plausible that investigation of the bacterial dysbiosis that arises in response to bariatric surgery may provide insight to: (1) novel approaches to inhibit pathways that promote disease progression in NAFLD, or (2) identification of non-invasive biomarkers to identify patients at risk for progression to NASH.

The only non-standard of care procedures that will occur during bariatric surgery is a liver biopsy for histology and exploratory analyses. In the post-surgery period, the only non-standard of care activities will be those related to the imaging and sample collection for monitoring of liver health with the specified biomarkers. Research-related procedures occurring before and after surgery are detailed below.

Study Agent, Device, and/or Intervention Description

To achieve one of our exploratory objectives, we will use the HepQuant SHUNT Liver Diagnostic Kit to quantitatively assess changes in liver function over time. The HepQuant SHUNT Liver Diagnostic Kit is an investigational device kit with an Investigational New Drug (IND) and Investigational Device Exemption (IDE) held by HepQuant, LLC. The US FDA originally in 2002 classified the HepQuant product as a drug. As such there are INDs for the drug components of the kit currently held by HepQuant, LLC: Cholic Acid-24-13C (IND # 65,121) and Cholic Acid-2,2,4,4-d4 (IND # 65,123). In 2015, the FDA re-classified the HepQuant SHUNT Liver Diagnostic kit as a diagnostic device and drug combination product. Therefore, the FDA requires that new clinical trials apply for an IDE, not an IND. Please refer to Investigator Brochure and email from manufacturer submitted with this package.

As sponsor of the IND and IDE, HepQuant, LLC. is responsible for FDA filings. Investigator compliance with Federal Food, Drug, and Cosmetic Act and the implementation regulations (Title 21 of the Code of Federal regulations, 21 CFR Part 50, 21 CFR Part 812, and 21 CFR Part 54) is defined in an Investigator Agreement with HepQuant, LLC. All investigational products will be stored in the secured pharmacy at the Translational Research Institute for Metabolism and Diabetes (TRI-MD). All products and procedures will be either handled and/or administered by trained clinical staff under the supervision of a study doctor.

All other interventions are either standard of care for bariatric surgery or listed below for research purposes.

Study Site(s)/Location(s) and Number of Subjects

AdventHealth Orlando site locations (campus, physician offices, etc.):

- Celebration Health
- Translational Research Institute for Metabolism and Diabetes

Estimated number of participants: 35

The study will enroll 25 participants with NASH for the full study and 10 participants with non-NASH [NAFLD or normal] to serve as controls. The 10 non-NASH participants will have baseline measurements and perioperative biopsies only. In the unlikely instance that biopsy results do not confirm NASH. Those participants who do not have NASH diagnosis will <u>not</u> continue in the post-surgery study visits and may become part of the 10 participants non-NASH group (see Figure 1 below). Additional participants may be recruited if the analysis of the primary end point (12 weeks) reveals larger variability than anticipated.

Multi-Site Research Logistics/Communication Plan

Not applicable.

Research Conducted in a Foreign Country

Not applicable.

Community-Based Participatory Research

Not applicable.

SUBJECT SELECTION

Vulnerable Populations

Not applicable.

Inclusion Criteria

To be eligible for this study, participants must meet the following inclusion criteria:

1. Age ≥18 years
2. Undergoing bariatric surgery (Roux-en-Y or gastric sleeve only) as part of clinical care.
3. Negative βhCG- level (female participants who become pregnant during the study will be withdrawn)
4. MRI:
   - for initial assignment to NASH cohort –
     - MRI-PDFF ≥8% (data generated by TRI-MD), and
     - magnetic resonance elastography (MRE) ≥3.00 kPa (Chen 2011, Dulai 2016, Loomba 2016, Costa-Silva 2018); or
     - MRE ≥ 2.70 kPa (Chen 2011) with at least one of the following risk factors for NASH:
       - type 2 diabetes
       - metabolic syndrome
       - ALT ≥ 1.5 times the upper limit of normal
       - Fibrosis-4 score suggestive of NASH (advanced fibrosis): Age ≤ 35: score not applicable; Age ≥ 36: > 2.67 (McPherson 2017)
       - AST to platelet ratio of ≥ 0.7, which is suggestive of NASH (significant fibrosis) (Lin 2011)
   - for initial assignment to NAFLD or normal participants cohort –
     - No requirement for minimum MRI-PDFF, and
     - MRE ≤ 2.50 kPa (Chen 2011, Dulai 2016, Loomba 2016, Costa-Silva 2018)

     Final Cohort assignment to be confirmed based upon results of liver biopsy (See Figure 1)
5. Histopathology assessment of liver biopsy that confirms NASH diagnosis

6. Hemoglobin A1c (HbA1c) ≤9.5%
7. Fasting triglycerides ≤400 mg/dL (4.5 mmol/L)
8. Body mass index (BMI) ≥35kg/m^2 (– 0.5 kg/m^2) when qualified for bariatric surgery
9. Able to speak and understand written and spoken English
10. Understands the procedures and agrees to participate by giving written informed consent
11. Willing and able to comply with scheduled visits, laboratory tests, and other study procedures

Exclusion Criteria

Exclude participants from this study if any of the inclusion criteria are not met, or if any of the following are observed:

1. Participation in other studies involving investigational drug(s) within **30 days** prior to Screening Visit 1
2. History of regular alcohol consumption exceeding 14 drinks/week for females or 21 drinks/week for males within the previous 6 months
   (1 drink = 5 ounces [150 mL] of wine, 12 ounces [360 mL] of beer, or 1.5 ounces [45 mL] of hard liquor)
3. A total score of ≥8 on the Alcohol Use Disorders Identification Test (AUDIT) questionnaire (Appendix B), indicating harmful or hazardous ethanol consumption
4. A positive urine drug test for illicit drugs
5. Clinical evidence of hepatic decompensation, including, but not limited to esophageal varices, ascites, or hepatic encephalopathy
6. Evidence of other forms of chronic liver disease (including laboratory tests as assessed by a sponsor-identified laboratory, and confirmed with a single repeat, if needed):
   - Hepatitis B virus (HBV): defined by presence of hepatitis B surface antigen (HBsAg)
   - Hepatitis C virus (HCV): As defined by a clinical history of previous diagnosis of Hepatitis C (treated or untreated) or a positive Hepatitis C antibody (HCVAb).
   - Human Immunodeficiency Virus (HIV) infection, defined as presence of HIV antibody
   - Known diagnosis of primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, or overlap syndrome
   - Alcoholic liver disease
   - Known diagnosis of hemochromatosis

- Prior known drug-induced liver injury
- Known or suspected hepatocellular carcinoma (HCC) or other liver cancer
- History of liver transplant, current placement on a liver transplant list, or current model of end-stage liver disease (MELD) score >12
- Histological presence of cirrhosis on a prior biopsy

7. Diagnosis of type 1 diabetes mellitus
8. Any malignancy not considered cured, except basal cell carcinoma and squamous cell carcinoma of the skin (a subject is considered cured if there has been no evidence of cancer recurrence in the previous 5 years)
9. Use of drugs historically associated with non-alcoholic fatty liver disease (NAFLD) for **≥1 month** in the previous year prior to Visit 3 (day of Surgery); examples include: amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, other known hepatotoxins
10. Subjects with history of severe claustrophobia impacting ability to perform MRI during the study without mild sedation/treatment with an anxiolytic
11. Subjects who fulfill any of the contraindications for MRI; examples include metal implants, devices, paramagnetic objects contained within the body and excessive or metal-containing tattoos
12. Unable to participate in MR assessments due to physical limitations of equipment tolerances (e.g., MRI bore size and 500-pound weight limit) based on Investigator's judgment at screening
13. Any person unable to lie still within the environment of the MRI scanner or maintain a breath hold for the required period to acquire images without mild sedation/treatment with an anxiolytic
14. Subjects with any anatomical or pathological abnormality that would either preclude or tend to confound the analysis of study data, including any clinically significant abnormal findings on the MRI obtained at Screening Visit 2.
15. Blood donation (excluding plasma donations) of approximately 1 pint (500 mL) or more within 56 days prior to Screening Visit 1 (participants may not donate blood any time during the study, through the final study visit)
16. Known history or suspected hypersensitivity to human serum albumin, or its preparations.
17. Investigator site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or subjects who are Pfizer employees, including their family members, directly involved in the conduct of the study
18. Participants who are NPO status will not receive the HepQuant test

19. Presence of any condition that, in the opinion of the Investigator, compromises participant safety or data integrity or the participant's ability to complete the study.

The eligible population for enrollment in the full longitudinal study is subjects with biopsy-confirmed NASH. Liver biopsies will be collected at the time of the bariatric surgery procedure; however, in advance of the surgery, screening criteria based on liver imaging assessments will be applied in a sequential manner in effort to help identify eligible subjects.

The MR assessment performed at Screening Visit 2 will serve as the non-invasive qualification of subjects with NASH, which will be defined as those having a liver fat-fraction ≥8% and liver stiffness ≥3.00 kPa or MRE ≥ 2.70 kPa (Chen 2011) with at least one of the following risk factors for NASH: type 2 diabetes, metabolic syndrome, Screening labs or labs drawn within 30 days prior to SV2 (ALT ≥ 1.5 times the upper limit of normal, AST to platelet ratio of ≥ 0.7, and/or Fibrosis-4 score suggestive of NASH: Age ≤ 35: score not applicable; Age ≥ 36: > 2.67 (Lin 2011, McPherson 2017). See Appendix C for description of how the scores are calculated.

The TRI-MD PDFF calculation will be used for determination of eligibility. Only enrolled participants will have their MRI data sent to AMRA and Perspectum for PDFF analysis during the study timepoints indicated in Appendix A: Schedule of Events. During the bariatric surgery procedure, subject inclusion will be confirmed following histological analysis of the liver biopsy. Following bariatric surgery, qualifying subjects will undergo periodic assessments out to 52 weeks post-surgery. In addition, subjects who either (1) do not meet the screening criteria for presumed NASH prior to surgery, or (2) meet the initial screening criteria, but do not meet the biopsy criteria for NASH diagnosis, will be considered for inclusion in a non-NASH cohort whose baseline assessments and perioperative biopsies will serve as control data, but for which no post-surgical longitudinal assessments will be performed.

A summary of the screening criteria for subject eligibility is provided in the decision tree diagram provided in Figure 1.

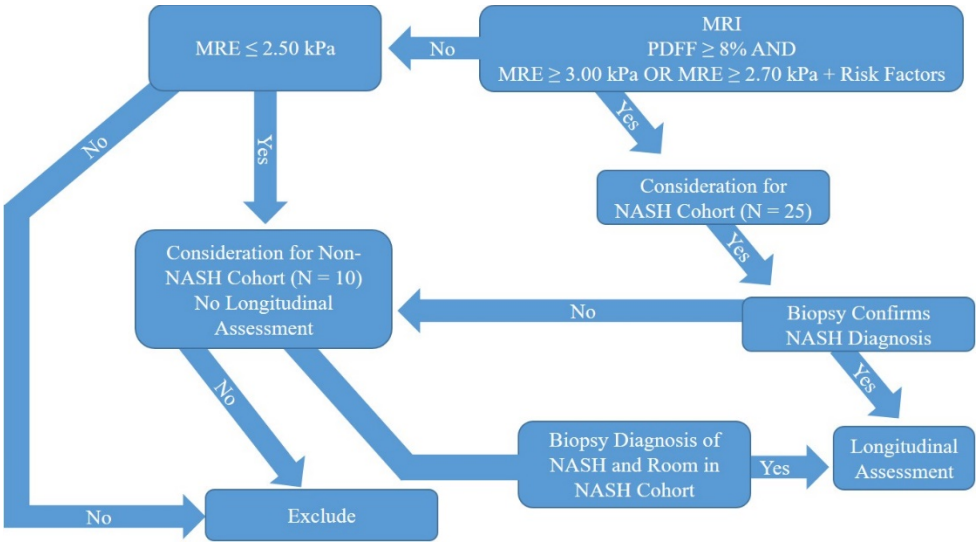

Figure 1. Decision Tree for Subject Eligibility

RESOURCES AVAILABLE

Site Initiation/Study Specific Training

All TRI-MD faculty and staff assisting with the research will be trained on the protocol design, study procedures, and their duties and functions pertaining to this study.
All training will be documented in accordance with AdventHealth Orlando ORA SOP 06 (Research Personnel Selection, Qualification, Responsibilities, and Training) and WI.031.50.001 Approval, Distribution and Training Process for standard operating procedures (SOPs), work instructions (WIs), and study-specific procedures (SSP)-TRI-MD. These polices include plans for ongoing discussion of issues throughout the duration of the study such as reportable new information, implementing amendments, study progress, etc.

STUDY PROCEDURES

Subject Recruitment and Screening

Recruitment methods utilized may include, but are not limited to, the following: electronic medical records searches; advertising in multiple media (such as print ads, flyers, brochures, posters); radio ads; television spots; and internet advertising (including social media). All advertising materials will be submitted to the Institutional Review Board (IRB) for review prior to using or publishing them.

Recruitment efforts will follow AdventHealth Orlando recruitment standard operating procedures (SOPs) for research. AdventHealth Orlando employees will not be individually targeted nor excluded from study participation based on employment. AdventHealth Orlando employees who engage the TRI-MD asking to participate in the study will be processed per standard consent procedures for participants. In addition, during the consent process, the study staff will review standard consent language stating that an employee's participation or lack of participation in the study will not affect their employment status or relationship with AdventHealth Orlando.

Consent Process

All study staff delegated the authority to obtain informed consent will follow HRP-802 (Investigator Guidance: Informed Consent) and HRP-803 (Investigator Guidance: Documentation of Informed Consent).

Non-English Speaking Subjects

Not applicable as the study will only target English-speakers.

Subjects who are Not Yet Adults (Infants, Children, Teenagers)

Not applicable.

Cognitively Impaired Adults

Not applicable.

Adults Unable to Consent

Not applicable.

Documentation of Informed Consent Process

Documentation of the informed consent process is required to establish that the participant was accurately and adequately informed and that no study-related procedures were initiated prior to obtaining informed consent. A research team member will note in the source documentation the consent process, the date consent was obtained, and that consent was obtained prior to initiating any research procedures.

Waiver of Written Documentation of Consent or Waiver of Consent

Not applicable.

Prohibitions and Restrictions

During study participation through the final study visit, subjects may not:

- donate blood
- use any investigational agent
- become pregnant (female subjects only)

Randomization

Not applicable.

STUDY VISITS

Procedures and assessments by visit are outlined below. For additional details and specific time points, see the Detailed Analytical/Clinical Procedures section and Appendix A: Schedule of Events, respectively.

Screening Visit 1 may occur during initial consultation at Celebration Health, or as a combination visit (SV1 and SV2) at TRI-MD. The surgery (Visit 3) will occur at Celebration Health, with all remaining visits occurring at TRI-MD.

Screening Visit 1 (SV1)

The following procedures may be performed per standard of care during or after the initial consultation visit for determination of eligibility for bariatric surgery. The data will be collected as part of this research study:

- physical examination
- height and body weight measured
- body mass index (BMI) calculated
- vital signs measured
- order provided for standard of care laboratory assessments
    - these tests may be repeated 2–3 weeks before Surgery

Signed informed consent will be obtained from interested participants who will then undergo the following procedures during their regularly scheduled initial consultation:

- assessment of participant eligibility
- demography collection
- adverse event (AE)/concomitant medication review

Screening Visit 2 (SV2)

This visit will occur as soon as possible after SV1, or as a combination with SV1, upon final determination of eligibility for bariatric surgery and based on convenience for participant. Any procedures required for eligibility that are not performed during SV1 will be done at SV2. The following procedures will be performed after an overnight fast:

- continued assessment of participant eligibility
- physical examination
- single supine vital signs
- height and body weight measured
    - Height will be repeated to ensure all future BMI comparisons are based on TRI-MD's calibrated scales and stadiometer.
- body mass index (BMI) calculated
- drug, alcohol, tobacco screen/AUDIT questionnaire
- AE/concomitant medication review
- blood collection for screening laboratories, including any the laboratory assessments at SV1 were not completed for any reason
- urinalysis
- urine pregnancy test
- urine drug screen
- FibroScan®
- MRI

Combination of Screening Visit 1 and Screening Visit 2 – if the initial surgery consultation at CH has been previously completed, SV1 and SV2 may be combined and completed at TRI-MD. All activities from SV1 and SV2 will be completed on the same day without duplicating activities.

Baseline Visit 1 (BLV1)

This visit will occur within 28 days after screening Visit 2 but prior the start of the standard of care pre-surgery liquid diet. The following procedures will be performed after an overnight fast:

- AE/concomitant medication review
- body weight measured
- BMI calculated
- single supine vital signs measured
- urine pregnancy test

- blood collection for hepatic panel
- blood collection for OWLiver Care, OWLiver, and comprehensive metabolomic panel from OWL Metabolomics
- blood collection for pro-collagen protein fragments panel from Nordic Bioscience
- HepQuant SHUNT Dual Cholate Liver Diagnostic test
- blood collection for biobanking
- stool collection during visit or with an at-home kit after the visit
- standard of care pre-surgery diet (will begin after Baseline Visit 1, but before Baseline Visit 2)

Baseline Visit 2 (BLV2)

This visit will occur within the final 5 days of the standard of care pre-surgery liquid diet. In the rare event that surgery is scheduled without sufficient time to collect BLV1 endpoints, participants can proceed to BLV2 and continue in the study. The following procedures will be performed after an overnight fast:

- AE/concomitant medication review
- body weight measured
- BMI calculated
- single supine vital signs measured
- urine pregnancy test
- blood collection for hemoglobin level
- blood collection for hepatic panel
- blood collection for OWLiver Care, OWLiver, and comprehensive metabolomic panel from OWL Metabolomics
- blood collection for pro-collagen protein fragments panel from Nordic Bioscience
- If BLV1 is not completed, stool collection will occur during this visit or with an at-home kit after the visit
- FibroScan®
- MRI

Surgery (V3) – Day 1

The following procedures will be performed as part of standard of care for bariatric surgery:

- body weight measured
- BMI calculated
- single supine vital signs measured
- urine pregnancy test
- Roux-en-Y or gastric sleeve will be performed using standard procedures by the bariatric surgeons at Celebration Health.

The following additional research procedures will be performed:

- Two liver biopsies for clinical histology and exploratory endpoints/biobanking

Post-Surgery Visits (V4) – Day 42 ±7 days (6 weeks)
(V5) – Day 84 ±7 days (12 weeks)
(V6) – Day 182 ±14 days (26 weeks)
(V7) – Day 364 ±14 days (52 weeks)

The following procedures will be performed after an overnight fast:

- AE/concomitant medication review
- body weight measured
- BMI calculated
- single supine vital signs measured
- urine pregnancy test
- blood collection for hepatic panel
- blood collection for OWLiver Care, OWLiver, and comprehensive metabolomic panel from OWL Metabolomics
- blood collection for pro-collagen protein fragments panel from Nordic Bioscience
- HepQuant SHUNT Dual Cholate Liver Diagnostic test
- blood collection for biobanking
- home stool collection during visit or with an at home kit after the visit
- FibroScan®
- MRI

DETAILED ANALYTICAL/CLINICAL PROCEDURES

Physical Examination and Medical History

A standard of care physical examination with collection of comprehensive medical history will be performed by a study physician, physician assistant, or nurse practitioner.

Drug, Alcohol, Tobacco Screen and AUDIT Questionnaire

Participants will be questioned regarding their consumption of illicit drugs, alcohol, and tobacco use. Participants will also complete the Alcohol Use Disorders Identification Test (AUDIT) questionnaire to screen for harmful or hazardous ethanol consumption. Appendix B shows the questions the participants will be asked along with the scoring methods; the latter will not be provided to participants.

Height, Body Weight, BMI Calculation

Body weight (calibrated scale) and height will be obtained while in a gown, with undergarments, without shoes. BMI will be calculated based on subject's body weight and standing height *[BMI = body weight in kg ÷ height in m^2]*.

Vital Sign Measurements

Single supine measurements of vital signs will include pulse/heart rate, systolic/diastolic blood pressure, respiratory rate, and oral/tympanic temperature. Pulse and blood pressure (using an appropriate size cuff for the subject's arm circumference) will be measured with participants in a supine position after 5 minutes of rest.

Screening Laboratory Assessments

The following laboratory assessments are utilized to assess eligibility during the screening process:

- complete blood count (CBC)
- platelet count
- comprehensive metabolic panel (CMP)
- lipid panel
- prothrombin (may be prothrombin time [PT]/international normalized ratio [INR]/partial thromboplastin time [PTT])
- activated partial thromboplastin time (APTT)
- HbA1c
- thyroid stimulating hormone (TSH) with reflex to thyroxine (T4)
- parathyroid hormone (PTH)
- urine pregnancy test
- urine drug test
- HIV antibody
- HBsAg (Hepatitis B antigen) screen
- HCVAb (Hepatitis C antibody)

These are used concomitantly to assess general patient health and determine study eligibility (Inclusion/Exclusion Criteria).

Circulating Biomarkers

Assessment of circulating biomarkers will be performed at scheduled visits as outlined in Appendix A: Schedule of Events.

Metabolomics: A comprehensive panel of serum metabolites will be measured at OWL Metabolomics. This panel of biomarkers allows for the identification and monitoring of disease progression / regression based on changes in the metabolomic signature of highly specific circulating lipid components. The panel includes OWLiver®, OWLiver®Care, and approximately 600 additional metabolites for exploratory purposes. This testing paradigm will allow for a comprehensive evaluation of changes in liver health and metabolic status after bariatric surgery/

Hepatic Panel: This panel includes total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). It is a standard clinical test commonly used to determine the health of the liver.

Pro-Collagen Protein Fragments: At minimum, we will measure two pro-collagen protein fragments at Nordic Bioscience- ProC3 and ProC6. Nordic Bioscience has developed a panel of biomarkers of extracellular matrix turnover that could provide important information about liver health including inflammation, disease prognosis, efficacy of interventions, and fibrogenesis.

We will bank additional samples for future assessment of novel biomarkers and mechanisms.

Details regarding type of sample, matrix, tubes, volume and sample management conditions for collection will be described in the Laboratory Manual.

Magnetic Resonance Imaging and Spectroscopy (MRI)

Assessment by MRI will be performed at scheduled visits outlined in Appendix A: Schedule of Events and will include measures of liver fat fraction, liver stiffness, liver relaxometry, and body composition. The goal of this test is to assess liver lipid content and stiffness, and perform volumetric fat quantitation using an Achieva 3T MRI/Multinuclear MRS (Philips, Andover, MA). Scans will be performed under standardized conditions with participants in a supine position, using the quadrature body coil and the torsoXL coil.

Standard MR images will be completed to obtain anatomical images for spectroscopy voxel and image slice localization and volumetric measurements of fat, muscle, organs, and bone in the participant's abdomen. Low resolution scans will be taken to determine appropriate positioning for high resolution images. Imaging sequences will then be performed over the participant's body, approximately spanning from the neck to the knees. The MRE driver will be placed on the participant's abdomen for the duration of the MR acquisition, and engaged during the MRE acquisition, for up to 3 total minutes.

Images which are degraded due to motion may be repeated as time permits.
The individual MRI scans and acquisition parameters are detailed in the Imaging Study-Specific- Procedure (SSP) and Scan Parameters documents. To minimize the potential for confounding factors to affect the measurements, MR scans will be conducted following a fast (except water) of at least 4 hours and as much as practically possible, at the same time of day relative to the screening assessment (±2 hours). Attempts will be made to ensure longitudinal assessment of an individual subject are performed by the same study staff member.

Total MRI scan time will be approximately 90 minutes.

For eligible participants at SV2, and BLV2, V4, V5, V6 and V7 as indicated in Appendix A: Schedule of Events, images will be sent for external analysis. Images will be sent to Advanced MR analytics AB (AMRA) for adipose tissue distribution analysis and steatosis quantification from the PDFF scans and to Perspectum Diagnostics for quantitative, multiparametric MRI analysis using LiverMultiScan, to determine their proprietary corrected T1 values, iron content, and liver steatosis.

FibroScan®

Approximation of liver fat and stiffness by FibroScan® will occur at scheduled visits outlined in Appendix A: Schedule of Events. Fibroscan® uses ultrasound based VCTE™ (vibration-controlled transient elastography) to obtain Liver Stiffness Measurement (LSM) and Controlled Attenuation Parameter (CAP™); LSM is correlated to liver

fibrosis stage, and CAP is correlated to hepatic steatosis. By using an ultrasound transducer probe, a painless, mechanical impulse is delivered to the skin above the liver using a low frequency elastic wave (50 MHz). The wave produces a mechanical deformation that moves towards and then through the liver. The velocity of the shear wave in the liver is measured and is directly proportional to its stiffness (stiffer tissue, faster velocity). Using a proprietary algorithm, the device determines whether the shear wave propagated into the liver and is valid. The LSM, expressed in kilopascals (kPa), is only calculated on valid measures. Liver stiffness measurement values range from 1.5 to 75 kPa; lower values indicate a more elastic liver. CAP™ measures the ultrasonic attenuation coefficient in the forward and return path through the liver at 3.5 MHz and is expressed in decibels/meter. CAP™ is evaluated simultaneously with LSM using the same radio-frequency data and is only appraised if the LSM is "valid." The specific FibroScan® model being utilized is the 502 Touch (Echosens, Paris, France).

Participants will lie on their back (supine) with their right arm raised in abduction to increase the intercostal space. A water-based gel will be applied to the skin and the transducer probe will then be placed on the participant's skin, in the intercostal space directly above the liver with slight pressure. At least ten valid measurements will be taken and the results will be immediate. To minimize the potential for confounding factors to affect the measurements, FibroScan® will be conducted following a fast (except water) of at least 4 hours and as much as practically possible, at the same time of day relative to the screening assessment (±2 hours). Only study staff members who are trained in the use of FibroScan® are permitted to acquire measures of liver stiffness and CAP™. Additionally, attempts will be made to ensure longitudinal assessments of an individual subject are performed by the same study site staff member.

The test takes a total of about 10 minutes, with total scan time (including setup) of approximately 30 minutes.

Details associated with the FibroScan® scanning protocol are documented in the Imaging SSP.

Quantitative Liver Function Test (HepQuant SHUNT Dual Cholate Liver Diagnostic Kit)

The HepQuant SHUNT Dual Cholate Liver Diagnostic Kit will be administered at scheduled visits outlined in Appendix A: Schedule of Events.

An indwelling peripheral venous catheter is used to collect a baseline Pre-Dose blood sample. Then, 20 milligrams of 13C-cholate is administered intravenously through the catheter, and 40 milligrams of d4-cholate is simultaneously administered orally, as detailed in the HepQuant Instructions for Use (IFU). Five timed blood samples drawn at 5, 20, 45, 60, and 90 minutes after the simultaneous dosing are utilized to measure the cholate pharmacokinetic (PK) clearances.

Total time for test setup and execution is expected to be 2 hours.

Additional details on sample processing are documented in the Laboratory Manual.

Liver Biopsy

The liver biopsy will be taken by the physician performing the bariatric surgery using a 14-gauge coring needle under direct visualization of the liver. The biopsy is obtained through an incision that is made for the placement of a liver retractor. The placement of the liver retractor is part of the standard procedure for bariatric surgery. Two small biopsy samples of the liver will be taken and then the biopsy site will be cauterized to stop any bleeding that occurred due to the procedure. One of the liver biopsies will be sent to a laboratory to be examined by a pathologist for diagnostic assessment of liver disease. Liver histology samples may be sent to a second blinded pathologist for research purposes. The second liver biopsy will be utilized for gene expression analysis via RNA sequencing (RNA-Seq). Any remaining tissue will be archived for future analyses.

A "Time Out" will be called during surgery where the surgeon and a research staff member will verify that the patient undergoing surgery is a research participant that is actively enrolled. The liver biopsy will not be collected for research purposes unless the time out is called.

Collection of the liver biopsies will add approximately 10 minutes to the usual bariatric surgery procedure.

Stool Collection

A stool sample will be collected at the following time points: Baseline visit 1, Day 42 ±7, Day 84 ±7, Day 182 ±14, and Day 364 ±14 from participants in the NASH cohort. Sampling will occur only at the pre-surgery time point in the non-NASH cohort. Participants will be asked to provide the stool sample during their regularly scheduled study visits. If the participant is not able to produce a sample, they will be provided with a stool specimen collection kit that includes all necessary supplies to collect the stool sample, a box with cold packs for transport and instructions for collection. Details of the stool collection kit and methods will be part of the an SSP and/or Laboratory Manual. Once produced, participants will call the study coordinator to make arrangements for the sample to be dropped off. If the participant is unable to drop off the stool sample, a courier will be set up to pick it up from their home.

STUDY DURATION

The overall study duration is anticipated to be up to 18 months. This duration assumes a period of several months between the first baseline visit and the second baseline visit, and the longitudinal study period will then continue for 12 months.

The study is projected to begin between May and July 2018, with study completion expected 1 year after recruitment of the final participant. Recruitment of all participants is anticipated to take approximately nine months.

MATERIALS OF HUMAN ORIGIN: COLLECTION, PREPARATION, HANDLING AND SHIPPING

All biological materials will be obtained per the procedures described in this protocol. For all study procedures, SSPs will be prepared or established SOPs or Work Instructions will be used. Samples will be collected and analyzed per the documented techniques established at the TRI-MD laboratory, Celebration Health and the AdventHealth Orlando Center for Diagnostic Pathology.

Biospecimen samples will be stored in ultralow temperature freezers and liquid nitrogen dewars or other storage units located at the TRI-MD Laboratory Room 2404. The TRI-MD facility is secured via key card and equipped with a back-up generator system. Laboratory personnel in the facility have 24/7 key-controlled access to the laboratory. Chain of custody of biospecimen samples is maintained through requisition forms and in the StarLIMS database. Specimen tubes are coded, and specimen requests and distribution are documented. Access to samples is limited to designated study team members as indicated on the delegation of authority log. Biological materials will be maintained in a locked and secured area within the TRI-MD facility. Physical access is limited by badge swipe assignments.

The biopsy that will be used for histology will be stored at the AdventHealth Orlando Center for Diagnostic Pathology for 10 years per the College of American Pathologists (CAP) protocol. Histology samples may be sent to a second pathologist for a blinded read for research purposes.

A subset of de-identified samples will be provided to our collaborators at Pfizer for future use as defined below.

Biospecimens collected for study-related endpoints, will be analyzed/tested at both AdventHealth Orlando and outside laboratories/institutions. Laboratories that will analyze de-identified samples may include, but is not limited to: HepQuant, OWL Metabolomics, Jackson Laboratories, and Nordic Bioscience.

Additionally, remaining biospecimens will be archived for any additional hypothesis-related experimentation or testing for this study, which could not be predicted at the time the protocol was developed.

Furthermore, if there are any left-over biospecimens after completing the above, they may be archived for the following: other research (not for this study), but of any type (without limitation to disease, process, or research methods). This other research can take place at AdventHealth Orlando or other institutions.

Lastly, a predetermined amount of biospecimen samples will be collected specifically for archiving for future use, such as other research (not for this study), of any type (without limitation to disease, process, or research methods). This other research can take place at AdventHealth Orlando or other institutions.

The biospecimens collected for this study will be separated into biospecimen samples that will be used for the study and biospecimen samples that were collected to be archived for future use. After study aims have been achieved and study related endpoints have been measured and analyzed, any remaining biospecimens will be stored at the

TRI-MD Laboratory Room 2404 and will also be considered as "archived biospecimens." Archived biospecimens will be used for any additional hypothesis-related experimentation or testing for the purposes of this study, consistent with the original aims, which cannot be predicted at the time the protocol is developed due to the evolving nature of scientific exploration.

Additionally, archived biospecimen samples may be stored indefinitely for future research. Archived biospecimens could be used for separate research by both AdventHealth Orlando scientists and scientists outside of AdventHealth Orlando. This would be allowed for research of any type (without limitation to disease, process, or research methods) if it has scientific merit as determined by the TRI-MD Scientific Review Committee. For research outside of AdventHealth Orlando, transfer and chain of custody of the biospecimens are detailed in the Contract with the Funder and in associated Vendor Agreements. In instances where these documents are not available, a Material Transfer Agreement (MTA) will be obtained, which will govern the transfer and chain of custody of the biospecimens outside of AdventHealth Orlando.

STUDY OUTCOME MEASURES (ENDPOINTS)

Please refer to Table 1.

DATA MANAGEMENT AND QUALITY PLAN

Data De-identification

Participants will be enrolled using Cerner's Patient Protocol Manager; the application assigns each participant a unique participant identifier, or "PID". This PID is a code consisting of a combination of numerals and letters, which serves as the identifier for this participant for this research study and links them back to their hospital medical record and their protected health information (PHI). Access to the "link" between the PIDs, the PHI, and to the clinical data are only granted to the clinical research team as assigned on the Delegation of Authority Log. All the clinical research data is recorded in a de-identified fashion onto our paper source documents, which is then transcribed into our electronic case report forms (eCRFs). The eCRF is used for storage (a database) and facilitates analysis. Clinical data generated by research devices also uses the PID, and once the data has been transformed into interpretable results, it is stored into the Clinical Research Database.Both storage locations are secured and only accessible to the assigned clinical research team. The "link" will only be used to re-identify participants in the event of a serious adverse event (SAE) requiring identification of a PID and PHI to treat the participant. The "link" will be stored in the Patient Protocol Manager and in Clinical Research Database, where only the TRI-MD research team has access. These secure databases are stored/accessed on the AdventHealth Orlando password-protected computer network. No one outside of AdventHealth Orlando investigators or researchers will have access to the databases.

Data Confidentiality, Storage, and Retention

The identity and personal health information will be kept confidential to the extent permitted by the applicable laws and/or regulations and will not be made publicly

available. If results of this study are published or presented, identities will not be revealed. Confidentiality will be maintained during and after the study. This information is also included in the informed consent, which is discussed with each participant prior to enrollment.

All paper sourced study documentation will be stored in the medical records room at TRI-MD, which is behind badge access doors, continuously locked, monitored and provides for limited access. The data records may also be stored as electronic records. This data is safeguarded so that only those on the research team have access to it via limited badge access to the medical records room (paper) or role relationship (electronic). The electronic data is maintained under Adventist Information Technology security controls.

The duration of study data retention will be determined by governing FDA regulations and/or sponsor contract mandate (if applicable). TRI-MD retention policy is maintained in the Records Management Policy. Electronic de-identified data will be kept indefinitely in our data warehouse.

Data Quality

Data quality and integrity will be governed by the system of ISO 9001 driven data management SOPs. These SOPs delineate how quality is built into the identification, collection, handling, and processing of the data.

The types of data collected may include data that are: (a) manually abstracted or electronically extracted from medical records, (b) observed in clinical exams, (c) obtained from laboratory and diagnostic tests, or from various biological monitoring or imaging devices, and (d) patient-reported items. Each data type can be understood by its source and the associated collection mechanism. Only the SOPs/SSPs and associated workflows applicable to the study will be used.

Within the study, quality control steps may include:

- Source Data Verification (SDV)
- Query Management
- Parallel Processing by a second party
- Complete re-processing of a percentage of the data and comparing to the original
- Change Control (if changes are made there will be documentation that covers change content, rationale, author, time and date)

Data Sharing (Outside of AdventHealth Orlando)

This is a AdventHealth-Orlando sponsored clinical study with Pfizer as the funder. De-identified results from this study will be shared with collaborators at Pfizer to conclude analysis and prepare data for publication. However, only AdventHealth Orlando will have access to the clinical data and the code to identify the samples and make the link between lab and clinical data. The source documentation will remain at TRI-MD.

Some of the endpoint testing and post-acquisition data analysis will be conducted at outside laboratories/institutions including, but not limited to, OWL Metabolomics, HepQuant, Nordic Bioscience, Jackson Laboratories, Perspectum Diagnostics

and AMRA. To perform these analyses/testing/etc. and to interpret results, certain data elements may need to be shared along with the biospecimen samples. The governance of data sharing is detailed in the Contract with the Funder and in associated Vendor Agreements. When these documents are not available, Data Use Agreements (DUAs) or Lab Services Agreements, as appropriate, will be obtained, which will identify the specific data elements to be shared (if any) and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by a study coordinator.

Should archived biospecimens be needed for research outside of AdventHealth Orlando that is beyond the details in the Contract with the Funder and in associated Vendor Agreements and certain data elements that are connected to the archived biospecimen samples are needed to conduct the research, then DUAs will be obtained. The DUAs will identify the purpose for data sharing, the specific data elements to be shared, and will govern the sharing of data related to this study. Data will be de-identified, however a link/code is managed within an electronic research management system and maintained by a study coordinator.

SAMPLE SIZE DETERMINATION

Sample size calculations are based on the two primary endpoints, percent change in PDFF and MRE. Based on a literature review it is assumed that the mean percent change from baseline in PDFF at 12–16 weeks has a standard deviation of 30%, the mean change from baseline in MRE has a standard deviation of 1 kPa. There is no formal hypothesis testing, however, the investigators would like to be able to detect a 30% change from baseline in PDFF and a 0.45 kPa change from baseline in MRE. Using a 1-sided t-test with an alpha level of 0.1, there is greater than 99% power for the PDFF change and greater than 80% power for MRE. Investigators are also interested in detecting a positive correlation between the 2 measures. A test for a correlation greater than zero, with 25 subjects, has greater than 80% power to detect a correlation of 0.42 or greater.

STATISTICAL ANALYSIS PLAN

The following subsection outlines the statistical methods that will be used in the analysis of the study data. Detailed descriptions will be included in the statistical analysis plan (SAP). Any deviations from the SAP will be described in the Clinical Study Report (CSR).

Summary Analyses

The number of subjects enrolled, completed, and discontinued from the study will be summarized. For subjects who did not complete the study, the reasons for withdrawal from the study will be presented.

Demographic and baseline characteristics will be summarized for all enrolled subjects. Continuous variables will be summarized using mean, median, minimum, maximum, quartiles, and standard deviation. Categorical variables will be summarized using relative frequency. Key demographic and baseline variables will be summarized and include, but are not limited to: age, gender, race, ethnicity, height, weight, BMI, etc.

Analysis of PDFF and MRE

Mixed model repeated measures analysis using all measured data will be used to explore changes over time, systematic differences, correlations with other endpoints, and time trends. The models will include baseline values of the measurements and other baseline demographic information as deemed necessary. Figures and tables will be presented. Details of the model and correlation structure will be detailed in the SAP.

Linear mixed models will be used at each time point to calculate the intra-class correlation coefficient between MRE and PDFF. The models will include baseline values of the measurements and other baseline demographic information as deemed necessary. The models will allow for different variance estimates for each test. Correlations, least squares means, and differences in least squares means, along with their corresponding confidence intervals and standard errors, will be presented in tabular form.

Imaging endpoints measured by other technologies as well as non-imaging endpoints will also be examined. Results will be tabulated and graphed as appropriate. Continuous variables will be summarized using mean, median, minimum, maximum, quartiles, and standard deviation. Categorical variables will be summarized using relative frequency. Mixed model repeated measures analyses using all measured data will be used to explore changes over time, systematic differences, correlations with other endpoints, and time trends. The models will include baseline values of the measurements and other baseline demographic information as deemed necessary. Intra-class correlations between endpoints may also be calculated. Details of the model and correlation structure will be detailed in the SAP.

Other exploratory and ad hoc analysis (if done) will be detailed in the CSR and/or SAP.

Multiplicity

Since this is an exploratory study intended to characterize PDFF and MRE measurements in a bariatric surgery population, no formal hypothesis testing is planned, therefore no multiplicity adjustments will be performed. P-values will be treated as descriptive statistics.

Missing Data

Descriptive summary statistics will use all available data. Repeated measures analyses will also use all available data and are expected to provide unbiased estimates under the assumption that data are missing at random (MAR) under the assumed model. Examination of informative missing data and sensitivity analyses may be performed if necessary and reported in the CSR. Multiple imputation (MI) may be used for sensitivity or secondary analyses.

Analysis Populations

All enrolled patients will be included in the full analysis set (FAS). The FAS will be used for all summaries and analyses unless otherwise specified in the SAP or CSR.

POTENTIAL RISKS AND BENEFITS

POTENTIAL BENEFITS

Participants will likely receive no direct benefit from taking part in this research study. However, the knowledge gained regarding liver disease in bariatric surgery patients may benefit public health as a whole. In addition, patients will receive information about their liver health before and after surgery, which may provide some benefit.

POTENTIAL RISKS

Intravenous Lines/Blood Draws (e.g., Laboratory Samples)

The placement of IV needles may cause transient pain, vasovagal syncope, and may also result in bruising, bleeding, and/or clotting at the site of needle insertion. The application of direct pressure at the catheterization site will be used to help prevent these symptoms. There is a possibility that a catheter placement would be unsuccessful or need to be removed. If this should occur, another catheter would be placed. It is possible that this may occur more than once during participation in the protocol. Staff who are trained and certified in the SOP will be used.

The total blood collected from each participant will be approximately 545 mL over a 52 week follow up period. This is inclusive of the approximately 90 mL collected at screening. The total blood volume within the first 8 weeks is approximately 280 mL.

Protection Against Risk

- All venipuncture will be conducted by qualified staff using aseptic techniques to reduce these risks.

Magnetic Resonance Imaging (MRI) and Spectroscopy

There are no known biological risks associated with MRI and spectroscopy. Some short -term discomfort may be experienced. The short-term risks associated with MRI are minimal, but include heating, loud noises, and claustrophobia. Some of the images require the use a soft rubber-like clip attached to your finger to monitor the heart beat in order to compensate for cardiac motion. There are some people who should not undergo MRI; the contraindication is largely based on the presence of metal objects within a person (i.e., pacemaker, aneurysm clip, metal fragments, etc.).

Protection Against Risk

- There will be strict safety screening per TRI-MD SOP 030.500.001 to ensure any people with contraindications are excluded from volunteering.
- Optional MRI Acclimatization:

  If a participant voices any concerns with tolerability of MRI scans, an acclimatization session may be performed during a screening visit. Participants will undergo an MRI acclimation session where they will be asked to lie in the MRI machine, in a manner similar to that required for MRI procedures in this study, to ensure participant

tolerability. This screening will last up to 5 minutes, and no data will be collected during this acclimation session.

Incidental Findings

There will be no diagnostic analysis associated with any of the MRI sequences used in this protocol. The images obtained at the screening visit will be sent for Radiology review to exclude any obvious medical abnormalities. In the case where an unexpected anatomical or pathological abnormality is reported by the Radiologist, the participant will be excluded from the study and instructed to seek medical attention from their health care provider. On subsequent study visits the images will not be sent for radiology review unless a medical abnormality is apparent on an image. In this case, the images will first be reviewed by an investigator on this protocol. If the abnormality is confirmed by radiology, then the participant will be instructed to seek medical attention from their health care provider. The results of the radiology interpretation will become part of the participant's medical record.

FibroScan®

There are no known risks associated with FibroScan®.

Protection Against Risk

Although there are no known risks, the FibroScan® test will be conducted only by individuals that have been trained and certified by the manufacturer.

Quantitative Liver Function Test (HepQuant SHUNT Liver Diagnostic Kit)

The following includes the known adverse events associated with the use of the HepQuant SHUNT Kit:

1. Risks from the Test Compounds
    a. Allergic reaction to cholate compounds (theoretical – none yet reported)
    b. Allergic reaction to human serum albumin (HSA)
    c. Reactions could include:
        i. rash
        ii. having a hard time breathing
        iii. wheezing when you breathe
        iv. sudden drop in blood pressure
        v. swelling around the mouth, throat, or eyes
        vi. fast pulse
        vii. sweating
        viii. severe reactions are very rare but a severe reaction (called anaphylaxis) can lead to profoundly low blood pressure and even death
2. Risks from the Indwelling Catheter
    a. Pain with placement of catheter
    b. Thrombosed vein
    c. Hematoma

Risks from the Test Compounds

Cholates, labeled with stable (nonradioactive) isotopes, occur naturally and are not known to have any deleterious or adverse effects when given intravenously or orally in the doses used in HepQuant tests. The serum cholate concentrations that are achieved by either the intravenous or oral doses are similar to the serum concentrations of bile acids that occur after the ingestion of a fatty meal. Because cholates are naturally occurring with a pool size in humans of 1 to 5 g, the 20 and 40 mg doses of labeled cholates used in the HepQuant tests are unlikely to be harmful to a fetus. However, the effects of these compounds on the fetus are not definitively known. Possible adverse event associated with the cholate test compounds may include: allergic reaction.

The two cholates used in the HepQuant test for this study are labeled with stable (non-radioactive) forms of carbon and hydrogen that are found in nature and can be measured in blood. These forms of cholate have been used with FDA Investigational New Drug (IND) research studies since 2002, and their use in humans has been monitored since that time. To date, the cholates used in this study have not been associated with any allergic reactions or side effects. However, they are still considered experimental and there may be unknown risks.

Human serum albumin is mixed with the ^{13}C-cholate for IV injection. Some individuals may have a known reaction to serum albumin, and will be excluded from this study. Possible AEs from the HSA may include: allergic reactions.

Rare Hypersensitivity Reactions have been reported to human serum albumin preparations and include anaphylaxis, severe anaphylaxis or anaphylactoid reactions, fever, chills, rash, urticaria, pruritus, angioneurotic edema, and erythema or flushing. Individuals who are hypersensitive to albumin preparations, any ingredient in the formulation, or components of the containers should be excluded from this substudy.

Risk of Transmissible Diseases in Plasma-derived Preparations. Because human albumin is prepared from pooled human plasma, there is a potential to pass human viruses (e.g., hepatitis viruses, HIV) to the recipient and may carry a risk of transmitting Creutzfeldt-Jakob disease (CJD) or its variant CJD (vCJD). Through donor plasma screening and specific procedures like pasteurization to eliminate or inactivate any possible causes have reduced, but not entirely eliminated, the risk of transmission of disease causing agents. Risk of transmission of viral disease with plasma-derived human albumin is considered extremely remote. No causes of transmission of HBV, HCV, or HIV have been documented following use of commercially available human albumin. There are no documented cases of CJD or vCJD transmitted through plasma-derived preparations (including plasma-derived human albumin); theoretical risk for transmission of CJD with commercially available human albumin is considered extremely remote.

However, no purification method has been shown to be totally effective in removing the risk of viral infectivity from plasma-derived preparations and because new blood-borne viruses or other disease agents may emerge which may not be removed or inactivated by current manufacturing processes, the risks of human albumin are not entirely known.

Risks from Indwelling Intravenous Catheter

Placing the indwelling intravenous catheter will cause minor pain and discomfort. With any blood draw, there is a small risk of hematoma and a very small risk of a blood clot (1 in 100) or infection (1 in 1000). HepQuant has performed the HepQuant SHUNT test or the prototypical dual cholate research test on over 500 individuals and most had the test multiple times. There has never been an adverse event (AE) - the risk from the test is very small. Possible adverse event from the placement of an indwelling catheter may include: hematoma at injection site.

Special Considerations in Bariatric Populations

After bariatric surgery, stomach capacity is approximately 30 milliliters. Sugared beverages are not well tolerated after bariatric surgery. It is recommended that 4 meals be consumed per day. Prolonged fasting periods may not be tolerated.

Other Considerations

Beta blockers or angiotensin converting enzyme (ACE) inhibitors could affect the blood flow to the liver, and therefore could affect the flow of the HepQuant SHUNT cholate test compounds into the liver as well. If the flow of blood to the liver was altered by these drugs, it could impact the SHUNT test results. Additionally, because individual participants may take different amounts of these drugs, corrections for the different concentrations and their impact on blood flow to the liver cannot be made. As such, participants who are currently taking either a beta blocker or an ACE inhibitor will be asked to delay taking their normal morning dose the day of their testing and until the 90-minute test is completed. Delaying these medications could cause a temporary elevation in blood pressure but the risk would be minimal, like that of participants that delay doses of medications in everyday life. Participants will therefore be instructed to immediately take their morning dose after the final 90-minute blood draw has been completed. Possible adverse events from delaying taking ACE inhibitor or beta blockers may include minimal change in blood pressure.

Protection Against Risk

- Individuals with a history or suspected hypersensitivity to human serum albumin, or its preparations will be excluded.
- The HepQuant test has not been used on individuals who have undergone bariatric surgery. Therefore, participants will be monitored closely. If there are issues with tolerability, the test will be stopped and no additional tests will be attempted. In addition, the following procedures will be implemented:
    - The total volume of the oral cholate mixture will be 20–30 milliliters.
    - Only artificially sweetened beverages will be used to prepare the cholate solution.
    - Participants will be able to consume clear liquids during the procedure to mitigate the effects of fasting.

Liver Biopsy

In most instances, there are no complications in obtaining a liver biopsy. Performing the biopsy does introduce the risk of bleeding as well as a bile leak from the biopsy site. It is also possible that inadvertent injury can occur to

surrounding organs from the biopsy needle. There are no separate skin incisions or punctures that are required for this procedure and there should be minimal to no added pain from the procedure.

Protection Against Risk

- Bleeding and bile leak risks are minimized by having the biopsy performed under direct visualization of the liver, which allows for the physician to confirm adequate hemostasis and the absence of a bile leak.
- Inadvertent injury to the surrounding organs from the biopsy need are minimized by having the biopsy conducted under direct visualization.
- A pathologist will review the results of the liver biopsy histology and the subsequent results and information will be available to the participant. Significant findings will be reported the bariatric surgeon. The results from this biopsy will become part of the participant's medical record.

Stool Collection

There are no known risks with collecting stool.

Protection Against Risk

N/A

MITIGATION OF RISKS

Risk monitoring will be performed via a weekly or biweekly individual review of cases by the Principal Investigator, the study physician, and/or research coordinator, including progress or AEs occurring in the following: subject confidentiality, subject recruitment, informed consent process, data quality, and any external factors relevant for the safety of participants throughout the entire study. In addition to these items, the study coordinator has been specifically instructed to make the Primary Investigator aware of all AEs, expected or unexpected that arise during the course of the study. Wound healing after invasive procedures (i.e., IV line placement) will be monitored. Laboratory tests are obtained during the screening visits of the study and any abnormal findings are sufficient to exclude individuals. If an abnormal laboratory value is obtained, the research participant will be referred to their primary care physician for follow-up. If illness or injury occurs during a study procedure, participants will be transported to the Emergency Room if needed.

Provisions to Protect the Privacy Interest of Subjects

Participants will be assigned unique identifiers for study-related records. All precautions will be taken to make sure that only authorized individuals will access subject research records. The collection of sensitive information about subjects will be limited to the minimum necessary to achieve the aims of the research, so that no unneeded sensitive information will be collected.

EARLY WITHDRAWAL OF SUBJECTS

Investigator Withdrawal of Subjects

Participation in the study may also be discontinued at any time at the discretion of the Principal Investigator, without participant consent if:

- The Principal Investigator believes it necessary for a participant's health or safety.
- Participant has not followed study instructions.
- Participant is not adherent to the intervention.
- The TRI-MD has stopped the study.
- Administrative reasons require a participant's withdrawal.

Participants will be withdrawn from the study if:

- Participant uses any investigational agent (up to the final study visit).
- Female participant has become pregnant.

Subject Request for Withdrawal from Study

Participation in this study is voluntary. Participants may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits. If a participant leaves the study before the final regularly scheduled visit, he/she may be asked by the Investigator to make a final visit for 'end-of-study' procedures. This is to ensure there are no safety concerns.

Data Collection and Follow-up for Withdrawn Subjects

If the study is prematurely terminated, or if a subject requests withdrawal from the study, or if the Principal Investigator withdraws a subject from the study, study data will be maintained in the research database up to the point of withdrawal. This data will be included in subsequent analysis, as keeping these participants in the analysis is essential for study validity.

ADVERSE EVENT REPORTING

Adverse Events

An AE is defined as both an expected side effect that is of a serious nature, or an unexpected side effect/event regardless of severity. Each participant is evaluated for AEs at every study visit. Any event that is reported to the study staff and meets the criteria of an AE will be documented as such and graded as to its attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol) and severity (mild, moderate, or severe). Any severe and/or unanticipated AE will be reported to the institutional review board (IRB) according to HRP 801 INVESTIGATOR GUIDANCE: Prompt Reporting Requirements.

Recording and Notification of Adverse Events

At each contact with the participant, the study team members will seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs

will be recorded immediately in the source document, and also in the appropriate AE module of the eCRF. All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document.

Any event meeting the reporting guidelines per HRP 801 INVESTIGATOR GUIDANCE: Prompt Reporting Requirements, will be reported to the AdventHealth Orlando IRB.

Safety Monitoring Plan

Safety Monitoring

Adverse events will be documented and reported by the study coordinator, the Principal Investigator, and other TRI-MD staff. Research and safety data will be reviewed by the Principal Investigator. This review will take place at regular meetings with the research coordinator and study physician where the safety labs for each new participant will be reviewed. Other items discussed will include progress or AEs occurring in the following: subject confidentiality, subject recruitment, and the informed consent process. All AEs and unexpected and/or severe AEs will be reported to the AdventHealth Orlando IRB per HRP 801 INVESTIGATOR GUIDANCE: Prompt Reporting Requirements. The TRI-MD has a standing committee that meets monthly to review all AEs in the clinical trials and will additionally be charged with review of the study.

Data and Safety Monitoring Board (DSMB) or Equivalent

Not applicable.

ETHICAL CONSIDERATIONS

Participation in this study is voluntary. Participants may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits. No vulnerable populations will be studied in this protocol.

Sharing of Results with Subjects

Participants will be offered the opportunity to meet with the Principal Investigator or designated medical staff to review the results of their laboratory assessments or other standard clinical data. Copies of their testing results will be made available to the participants upon request. In addition, the Principal Investigator or designated study staff will provide an overview of the study's outcome to the participant, when available, if he or she requests the information.

FUNDING SOURCE

This study will be externally funded by Pfizer Inc.

PARTICIPANT STIPENDS OR PAYMENTS

After the participant’s completion of all study visits, a total amount for the study will be $1,000-1,375.00. A Mastercard® payment will be processed with the Dollar amount per visits completed as indicated in the table below. Mastercard® payments may take up to 3 business days to be

processed, once requested. In the event the participant is unable to complete all study visits the payment will be prorated.

The payments are broken into two payments: Screening Visit 1 through Visit 5 (after completion of all visits through the 12-week time point), and Visits 6-7 (after completion of the remaining visits ending at the 52-week time point).

Compensation for Baseline Visit 1 and Visits 4-7 will vary, depending whether a stool sample is provided and the dual cholate test is completed.

Participants who agree to take part in this study will be paid for completed procedures according to the following schedule:

Visit	Total
***Screening* Visit 1**	*$50.00*
***Screening* Visit 2** **If both screening visits are combined, the payment will also be combined**	*$125.00*
Baseline Visit 1 **Stool - $25.00; Dual cholate $50.00**	*$50.00-125.00*
Baseline Visit 2	*$125.00*
Visit 3 (Surgery)	*$150.00*
Visit 4 **Stool - $25.00; Dual cholate $50.00**	*$125.00-200.00*
Visit 5 **Stool - $25.00; Dual cholate $50.00**	*$125.00-200.00*
Visit 6 **Stool - $25.00; Dual cholate $50.00**	*$125.00-200.00*
Visit 7 **Stool - $25.00; Dual cholate $50.00**	*$125.00-200.00*
TOTAL	***$1,000-1,375.00***

PUBLICATION PLAN

TRI-MD faculty and staff will adhere to POLICY-TRI-ADM-005 (Access to Clinical Trial Data for Publication Purposes).

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APPENDIX A. SCHEDULE OF EVENTS

Description	Screening Visit 1	Screening Visit 2	Baseline Visit 1	Baseline Visit 2	Surgery	Post-Surgery			
Visit Number	**SV1**	**SV2[l]**	**BLV1[m]**	**BLV2**	**V3**	**V4**	**V5**	**V6**	**V7**
Day [a]	**Varies**	**Varies**	**Varies**	**Varies**	**1**	**42 ±7**	**84 ±7**	**182 ±14**	**364 ±14**
Physical Exam [b]	X	X							
Provider time to explain study	X								
Assess participant eligibility	X	X			X				
Informed consent [c]	X								
Demography [c]	X								
AE/ConMed review		X	X	X		X	X	X	X
Drug Alcohol Tobacco Screen/AUDIT questionnaire		X							
Height and body weight [d]	X	X	X	X	X	X	X	X	X
Body Mass Index (BMI)	X	X	X	X	X	X	X	X	X
Vital signs [b]	X	X	X	X	X	X	X	X	X
Screening Lab assessments (fasting) [e]		X							
Urine pregnancy test		X	X	X	X	X	X	X	X
Urinalysis		X							
Urine drug screen		X							
HIV, HBV, HCV screen		X							
Hepatic panel			X	X		X	X	X	X
Hemoglobin				X					
Pre-surgery diet [f]				X					
SoC Bariatric surgery					X				
Liver biopsy [g]					X				
Blood collections for: OWL Metabolomics Nordic Bioscience			X	X		X	X	X	X
Blood collections for: Biobanking [h]			X			X	X	X	X
Stool Collection [i]			X			X	X	X	X
HepQuant SHUNT Dual Cholate Liver Diagnostic Kit [j]			X			X	X	X	X
IMAGING									
FibroScan®		X		X		X	X	X	X
MRI[k]		X		X		X	X	X	X

SCHEDULE OF EVENTS, cont.

Abbreviations: AE = adverse event; AUDIT = Alcohol Use Disorders Identification Test; CH = Celebration Health; ConMed = concomitant medication; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; MRE = magnetic resonance elastography; MRI = magnetic resonance imaging; SoC = standard of care; TRI-MD = Translational Research Institute for Metabolism and Diabetes.

Note: Screening Visit 1 (SV1) may occur either at CH or TRI-MD. Surgery (V3) occurs at CH. All other visits and procedures, including the combination screening visit (if applicable), occur at TRI-MD.

a. Pre-surgery time points will vary based upon insurance requirements, length of diet, and other clinical needs.
b. Performed by CH provider as a part of standard of care procedures at SV1 and at TRI-MD at indicated visits per our standard research procedures.
c. Performed by CH study coordinator/CH provider.
d. Height measured at SV1 and SV2 only; weight measured at designated visits for calculation of body mass index (BMI).
e. Screening laboratory assessment are collected at SV2 or the combination SV1/SV2 visit and include: complete blood count (CBC), platelet count, comprehensive metabolic panel (CMP), lipid panel, prothrombin (may include prothrombin time [PT]/international normalized ratio [INR]/partial thromboplastin time [PTT]), activated partial thromboplastin time (APTT), , hemoglobin A1c (HbA1c), thyroid stimulating hormone (TSH) with reflex to thyroxine (T4), parathyroid hormone (PTH).
f. The pre-surgery diet varies and occurs as per standard of care. BLV2 will occur during the last 5 days of the liquid diet.
g. Liver biopsy for clinical histology and research. Participants without non-alcoholic steatohepatitis (NASH) upon biopsy will not continue longitudinal assessment. The Liver Biopsy is the final criteria determining the cohort (NASH vs NON-NASH)
h. Blood collections for biobanking: plasma, serum, PAX DNA, P800 tube.
i. Stool will be collected during clinic visit if possible. If not, a home stool collection kit will be sent home with participant.
j. HepQuant SHUNT Dual Cholate Liver Diagnostic will be discontinued if there are any feasibility or tolerability issues.
k. The TRI-MD liver fat percentage by Proton Density Fat Fraction (PDFF) will be used to ascertain eligibility. For participants that meet eligibility criteria at SV2, data will be sent to Perspectum and AMRA to generate PDFF and other liver endpoints from MRI data collected at SV2 and BLV2, V4, V5, V6 and V7.
l. SV2 will occur as soon as possible after SV1.
m. BLV1 will occur within a maximum of 28 days from SV2.

APPENDIX B. ALCOHOL USE DISORDERS IDENTIFICATION TEST (AUDIT) QUESTIONNAIRE

Please circle the answer that is correct for you

1. How often do you have a drink containing alcohol? Never Monthly or less 2–4 times a month 2–3 times a week 4 or more times a week	**6. During the past year, how often have you needed a drink in the morning to get yourself going after a heavy drinking session?** Never Less than monthly Monthly Weekly Daily or almost daily
2. How many standard drinks containing alcohol do you have on a typical day when drinking? 1 or 2 3 or 4 5 or 6 7 to 9 10 or more	**7. During the past year, how often have you had a feeling of guilt or remorse after drinking?** Never Less than monthly Monthly Weekly Daily or almost daily
3. How often do you have six or more drinks on one occasion? Never Less than monthly Monthly Weekly Daily or almost daily	**8. During the past year, have you been unable to remember what happened the night before because you had been drinking?** Never Less than monthly Monthly Weekly Daily or almost daily
4. During the past year, how often have you found that you were not able to stop drinking once you had started? Never Less than monthly Monthly Weekly Daily or almost daily	**9. Have you or someone else been injured as a result of your drinking?** No Yes, but not in the past year Yes, during the past year
5. During the past year, how often have you failed to do what was normally expected of you because of drinking? Never Less than monthly Monthly Weekly Daily or almost daily	**10. Has a relative or friend, doctor or other health worker been concerned about your drinking or suggested you cut down?** No Yes, but not in the past year Yes, during the past year

Scoring the AUDIT

Scores for each question range from 0 to 4, with the first response for each question (e.g., never) scoring 0, the second (e.g., less than monthly) scoring 1, the third (e.g., monthly) scoring 2, the fourth (e.g., weekly) scoring 3, and the last response (e.g., daily or almost daily) scoring 4. For questions 9 and 10, which only have three responses, the scoring is 0, 2 and 4 (from left to right).

A score of 8 or more is associated with harmful or hazardous drinking, a score of 13 or more in women, and 15 or more in men, is likely to indicate alcohol dependence.

Saunders JB, Aasland OG, Babor TF, et al. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption — II. *Addiction.* 1993; 88: 791–803.

APPENDIX C. Liver Disease Score Calculation

To further evaluate potential risk of NASH, we will calculate two different scores based on bloodwork and clinical data we are already collecting.

The AST to Platelet Ratio (APRI) score is calculated via the following formula:
(AST in IU/L) / (AST Upper Limit of Normal in IU/L) / (Platelets in 10^9/L)

The Fibrosis-4 (FIB-4) Index for Liver Fibrosis is calculated with the following formula:
(Age x AST) / (Platelets x √(ALT))