

Inclusion and Exclusion Criteria

Inclusion Criteria

1. Age 18-65 years
2. Weight stable (weight change of no more than 3 kg + 0.5 kg) during the 6 months prior to enrollment
3. Fasting triglycerides ≤ 400 mg/dL
4. Body mass index (BMI) 25-39.9 kg/m²
5. Able to speak and understand written and spoken English
6. Understands the procedures and agrees to participate by giving written informed consent
7. Willing and able to comply with scheduled visits, laboratory tests, and other study procedures
8. MRI-PDFF Criteria ⁶⁵:
 - a. Controls: $< 5\%$
 - b. Steatosis Only: $\geq 5.6\%$
 - c. Fibrosis: no specific percentage required
9. MRE Criteria:
 - a. Controls: < 2.50 kPa ^{66,67}
 - b. Steatosis Only: < 2.50 kPa ^{66,67}
 - c. Fibrosis (without cirrhosis): $> 2.61 - < 4.69$ kPa ⁶⁸; if liver histology data is available within 12 months of screening, this can be used instead of MRE results to confirm presence of fibrosis.

Exclusion Criteria

Acute or chronic medical conditions or medication that would contraindicate the participation in the research testing or could potentially affect metabolic function including, but not limited to:

1. Diagnosis of type 1 or type 2 diabetes mellitus
2. Insulin use
3. Treatment with pioglitazone or metformin
4. 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)
5. A total score of ≥ 8 on the Alcohol Use Disorders Identification Test (AUDIT) questionnaire, indicating harmful or hazardous alcohol consumption
6. Clinical evidence of hepatic decompensation, including, but not limited to esophageal varices, ascites, or hepatic encephalopathy.
7. Evidence of other forms of chronic liver disease (including laboratory tests and confirmed with a single repeat, if needed):
 - a. Hepatitis B virus: defined by presence of hepatitis B surface antigen
 - b. Hepatitis C virus: As defined by a clinical history of previous diagnosis of Hepatitis C (treated or untreated) or a positive Hepatitis C antibody.
 - c. Known diagnosis of primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, or overlap syndrome
 - d. Alcoholic liver disease
 - e. Known diagnosis of hemochromatosis
 - f. Prior known drug-induced liver injury
 - g. Known or suspected hepatocellular carcinoma or other liver cancer
 - h. History of liver transplant, current placement on a liver transplant list, or current model of end-stage liver disease (MELD) score > 12
 - i. Histological presence of cirrhosis on a prior biopsy
8. Bleeding disorders
9. Current treatment of blood thinners or antiplatelet medications that cannot be safely stopped for biopsy procedure.
10. Acute or chronic infections
11. Severe asthma or chronic obstructive pulmonary disease
12. Renal insufficiency or nephritis

13. Thyroid dysfunction (suppressed TSH, elevated TSH <10 µIU/ml if symptomatic or elevated TSH >10 µIU/ml if asymptomatic)
14. Uncontrolled hypertension (BP >160 mmHg systolic or >100 mmHg diastolic)
15. Prior or planned bariatric surgery
16. Gastrointestinal disorders including: inflammatory bowel disease or malabsorption, swallowing disorders, suspected or known strictures, fistulas or physiological/mechanical GI obstruction, history of gastrointestinal surgery, Crohn's disease or diverticulitis.
17. A positive urine drug test for illicit drugs
18. History of major depression within < 5 years from screening or which, in the opinion of a medical provider, will impact the participant's ability to complete the study.
19. History of eating disorders
20. History of Cushing's disease or syndrome
21. Active rheumatoid arthritis or other inflammatory rheumatic disorder
22. Pregnant or nursing females or females less than 6 months postpartum from the scheduled date of Biopsy Visit.
23. Nicotine use within the past 3 months
24. Major surgery within 4 weeks prior to Screening.
25. Anemia (hemoglobin <12 g/dl in men, <11 g/dl in women) during screening
26. Participation in studies involving investigational drug(s) within 30 days prior to Screening
27. History or presence of cardiovascular disease (unstable angina, myocardial infarction or coronary revascularization within 6 months, presence of cardiac pacemaker, implanted cardiac defibrillator)
28. Human Immunodeficiency Virus (HIV) infection defined as: previous diagnosis of HIV infection, history of positive screening or quantitative HIV testing; positive HIV screen
29. Any malignancy not considered cured, except basal cell carcinoma and squamous cell carcinoma of the skin (a participant is considered cured if there has been no evidence of cancer recurrence in the previous 5 years)
30. Use of antibiotics within 3 months of screening
31. Use of drugs historically associated with non-alcoholic fatty liver disease (NAFLD) for ≥1 month in the previous year prior to Screening; 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
32. Participants 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
33. Unable to participate in MR assessments due to physical limitations or equipment tolerances (e.g., MRI bore size and 500-pound weight limit) based on Investigator's judgment at screening
34. Any person with history of severe claustrophobia or unable to lie still within the environment of the MRI scanner or unable maintain a breath hold for the required period to acquire images without mild sedation/treatment with an anxiolytic
35. Blood donation (excluding plasma donations) of approximately 1 pint (500 mL) or more within 56 days prior to Screening (participants may not donate blood any time during the study, through the final study day)
36. Presence of any condition that, in the opinion of the Investigator, compromises participant safety or data integrity or the participant's ability to complete study days.