Development and evaluation of a glucagon sensitivity test in individuals with and without hepatic steatosis (GLUSENTIC).

NCT number: NCT04907721

Date: May 17th, 2021 (latest revision: September 19th, 2023)

STATISTICAL ANALYSIS PLAN

Ethical Approval number: H-20023717

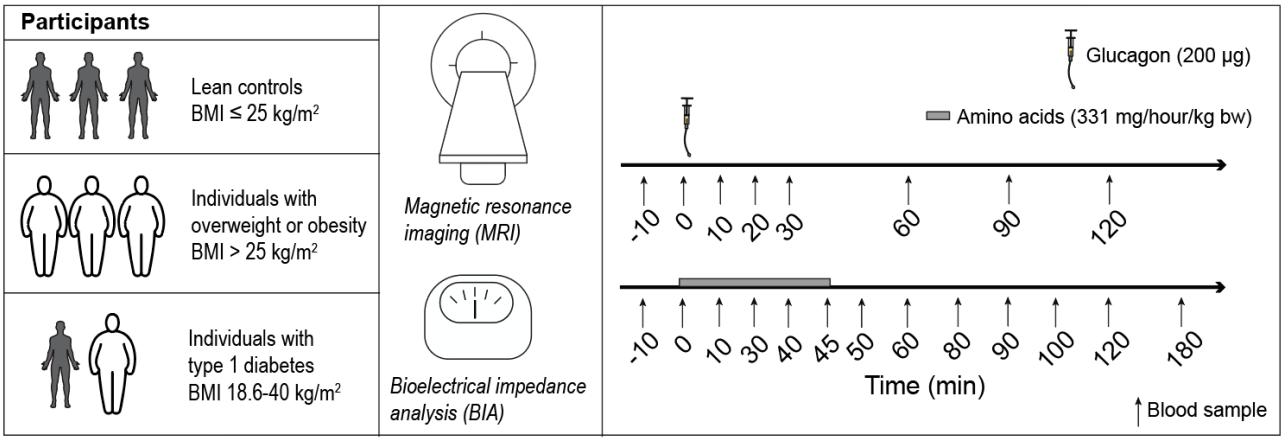
Principle investigators: Nicolai J Wewer Albrechtsen and Jørgen Rungby.

Co-investigators: Sasha A S Kjeldsen, Jens Juul Holst, Nicole J Jensen, Michael M Richter, Malin S D Nilsson, Mikael P Boesen, Janus D Nybing, Steen B Haugaard, Sten Madsbad, Lise Lotte Gluud, Hendrik Vilstrup, Kirsten Nørgaard, Signe Schmidt.

Aim of study: This is a cross-sectional study investigating the connection between metabolic dysfunction-associated steatotic liver disease (MASLD) and glucagon's capacity to metabolize amino acids. We will recruit the following three groups of individuals:

1. Lean individuals (BMI < 25 kg/m^2; n=20)
2. Overweight and obese individuals (BMI ≥ 25 kg/m^2; n=30)
3. Individuals with type 1 diabetes (BMI 18.6-40 kg/m^2; n=15)

Groups will take part in two experimental trial days:



The two groups of 'lean controls' and 'individuals with overweight or obesity' will subsequently be stratified into two new groups based on hepatic steatosis measured by magnetic resonance imaging. Individuals with <5.6 % hepatic steatosis will be stratified into the control group and individuals with ≥5.6% hepatic steatosis will be stratified into the MASLD group. Participant with type 1 diabetes will remain a separate group.

Endpoints

Primary outcome:

Differences in the calculated GLUSENTIC index between individuals with (>5.6%) (MASLD) or without (controls) hepatic steatosis. Patients with type 1 diabetes will not be included in the primary outcome.

The calculated GLUSENTIC index for glucagon sensitivity is based on the following equation (1):

$$\frac{100}{SQRT(fAA * aGCG * sAA * sGCG)}$$

fAA = Fasting plasma amino acids (mean value at time -10 and 0).
fGCG = Fasting plasma glucagon (mean value at time -10 and 0).
sAA = Plasma amino acids during the amino acid stimulation (mean value at time 40 and 45).
sGCG = Plasma glucagon during the amino acid stimulation (mean value at time 40 and 45).

The GLUSENTIC index is conceptually based on the Matsuda/composite index (2, 3).

Secondary outcomes:

The following secondary endpoints will be compared between controls (hepatic steatosis <5.6%), MASLD (hepatic steatosis ≥5.6%) and individuals with type 1 diabetes using one-way ANOVA unless otherwise stated. The control group will be used as reference in the statistical analysis. Differences in plasma levels of insulin and C-peptide between individuals with or without hepatic steatosis will be assessed by t-test.

1) Simple linear regression between the variables, hepatic steatosis and the GLUSENTIC index in individuals without diabetes
2) ROC curve analysis to evaluate a cut-off value for the GLUSENTIC index
3) Differences in the glucagon-alanine index.
4) Differences in plasma levels of amino acids during the amino acid tolerance test (determined by baseline corrected AUC)
5) Glucagon's ability (exogenous glucagon) to increase amino acid disappearance (determined by baseline corrected AUC or delta) for total amino acid levels and the individual amino acids.
6) Differences in plasma levels of glucagon, total amino acids, and alanine following an overnight fast.
7) Baseline corrected AUC for glucagon, glucose, insulin, C-peptide, urea, and triglycerides after a bolus injection of glucagon.
8) Baseline corrected AUC for glucagon, glucose, insulin, C-peptide, urea, and triglycerides during and after the amino acid tolerance test.
9) Differences in the formula (plasma urea/plasma amino acids) after the amino acid tolerance

test.

10) Differences in amino acids, alanine, and glucagon between individuals with overweight or obesity without hepatic steatosis who have been BMI-matched to individuals with hepatic steatosis.

11) Simple linear regression between the variables, pancreatic steatosis and amino acid stimulated glucagon or insulin levels in individuals without diabetes.

Explorative outcomes:

Differences in plasma Lp(a), GLP-1, GIP, hepatokines (including FGF21, GDF-15, fetuin A, fetuin B, follistatin and FGL1), lipid-species, plasma proteomics and focused genotyping (on the specific variants in PNPLA3; MBOAT7; GCKR; TNF; TM6SF2). These data will not be reported in the first primary article.

1. Kjeldsen SAS, Richter MM, Jensen NJ, Nilsson MSD, Heinz N, Nybing JD, et al. Development of a glucagon sensitivity test in humans: Pilot data and the GLUSENTIC study protocol. *Peptides.* 2023;161:170938.
2. Matsuda M, and DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* 1999;22(9):1462-70.
3. DeFronzo RA, and Matsuda M. Reduced time points to calculate the composite index. *Diabetes Care.* 2010;33(7):e93.