# Statistical Analysis Plan

## EndoBarrier™ in obese subjects with type 2 diabetes: Impact on pancreatic function, insulin resistance, gut peptides, and gut permeability – a pilot study

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# Introduction

Obesity has reached epidemic proportions in the western world and has risen to the top of the public health policy agenda. The World Health Organisation (WHO) predicts that approximately 2.7 billion adults will be overweight (Body mass index (BMI) 25-29.9 kg/m²) by 2025, and worldwide, more than 177 million adults will be severely obese and in need of treatment (BMI 35 kg/m² or more )[1].

The association between obesity and type 2 diabetes mellitus (T2DM) is well recognised. A higher BMI is a strong predictor for T2DM[2]. Currently, 285 million people suffer from T2DM, and this number is anticipated to increase to 439 million by 2030[3]. T2DM raises the risk for cardiovascular events, heart failure, eye problems, and chronic kidney disease. Previous research has demonstrated that the impact of diabetes on daily life and the likelihood of costly and disabling complications can be minimized by more intensive management of glucose[4], blood pressure[5], and cholesterol[6].

Bariatric surgery (BS) was established as a treatment option for morbidly obese or obese people with comorbidities such as T2DM. Besides reducing body weight, BS was reported to improve glycaemic control in patients with T2DM and importantly reduces cardiovascular events and overall mortality in obese subjects[7-10].

It was demonstrated that glycaemic improvement occurs rapidly within the first weeks after BS and cannot be explained by the relatively small weight loss occurring during this short period. These beneficial glycaemic changes after bypass procedures were attributed to changes in incretin levels and the interplay of other gut hormones[11].

In 2010, a duodenal-jejunal bypass liner (DJBL, Endobarrier™, GI Dynamics, Lexington, Massachusetts, USA) was introduced and CE marked. The EndoBarrier™ is a 60 cm long impermeable fluoropolymer sleeve-like device, which is placed endoscopically via oral route and anchored in the duodenum creating a duodenal-jejunal bypass for a maximum of 12 months. It allows transit of chyme from the stomach through the jejunum without any contact to the duodenal mucosa. Both bile and pancreatic exocrine secretions do not get in contact with chyme in the proximal jejunum, thus the DJBL mimics a duodenal-jejunal bypass and therefore induces weight loss.

A number of studies demonstrated the feasibility of implanting this device with subsequent weight reduction[12-16]. In T2DM subjects, significant improvements in glycaemic parameters after EndoBarrier™ implantation were observed as well as an improvement of insulin resistance measured by Homeostatic model assessment – insulin resistance (HOMA-IR) [17, 18].

However, thorough investigation of insulin resistance and beta-cell function with the gold standard, namely an intravenous glucose tolerance test combined with a hyperinsulinaemic-euglycaemic clamp (Botnia clamp) is lacking so far. The aim of the current study is to explore short and longer-term effects (26 weeks after removal) of the EndoBarrier™ implantation on insulin resistance and beta-cell function assessed by repeated Botnia clamps to further delineate mechanisms of this intervention.

As the ENDO trial investigating the effects of the Endobarrier™ device compared to a sham intervention was terminated early due to a higher rate of hepatic abscesses than expected, speculations about reasons are ongoing. A leaky gut and changes in the upper gastrointestinal microbiome caused by the bypass-liner are discussed. Therefore, another aim of this study is to investigate potential changes in microbiome composition and gut permeability.

# Research objectives

## Primary objectives

The primary objective of this trial is to determine the changes in insulin sensitivity and beta-cell function after EndoBarrier™ implantation during a follow up of a 36-week period in patients with T2DM and a BMI between 30.0 and 49.0 kg/m$^2$.

## Secondary objectives

1) To assess the beta cell insulin response to oral and intravenous glucose loads early (4 weeks) and 36 weeks after EndoBarrier™ implantation as well as 26 weeks after removal of the duodeno-jejunal bypass device.
2) To assess changes in body weight from baseline to 4 weeks, 36 weeks and 62 weeks, respectively.
3) To determine changes in gut permeability and gut microbiome composition from baseline to 4 weeks, 36 weeks and 62 weeks, respectively.
4) To evaluate changes in area under curve (AUC) of glucose from baseline to 4 weeks, 36 weeks and 62 weeks, respectively.
5) To assess changes in body composition assessed by Dual-energy X-ray absorptiometry (DEXA) from baseline to 4 weeks, 36 weeks and 62 weeks, respectively.
6) To evaluate correlations between mixed meal tolerance test (MMTT) and Botnia clamp results regarding insulin sensitivity and beta-cell function.
7) To determine changes in cardiovascular risk as assessed by the United Kingdom Prospective Diabetes Study (UKPDS) risk engine from baseline to 4 weeks, 36 weeks and 62 weeks, respectively.

# Study Methods

# Inclusion and Exclusion Criteria

## Inclusion criteria

- Participant is willing and able to give informed consent for participation in the study.
- Male or female
- Age: 18 - 70 years
- Type 2 diabetes
- BMI 30-49 kg/m²
- HbA1c ≥ 6.5% (48 mmol/mol)
- Appropriate lifestyle intervention measures have been tried but have failed to achieve or maintain adequate, clinically beneficial weight loss for at least 6 months
- Person is generally fit for intervention
- Person commits to the need for long-term follow-up

## Exclusion criteria

- Type 1 diabetes mellitus
- Maturity Onset Diabetes of the Young (MODY)
- Secondary diabetes due to a specific disease or glucocorticoid therapy
- Pregnancy or women of childbearing age without adequate contraception
- Women who are breast-feeding
- Hypothalamic cause of obesity, Cushing syndrome
- Major psychiatric disease including diagnosed eating disorders, history of drug or alcohol abuse
- History of bariatric surgery or complex abdominal surgery
- Inflammatory bowel disease
- Pancreatitis
- Cholelithiasis
- Uncontrolled gastroesophageal reflux
- Known upper GI bleeding conditions, e.g. gastric or esophageal varices
- Congenital or acquired abnormalities of the upper GI tract, e.g. stenosis
- Subjects with or a history of coagulopathy, upper gastro-intestinal bleeding conditions such as esophageal or gastric varices, congenital or acquired intestinal telangiectasia
- Chronic non-steroidal anti-inflammatory drug (NSAID) or aspirin treatment (Subjects unable to discontinue NSAIDs (non-steroidal anti-inflammatory drugs) during the implant period)
- Previous GI surgery that could affect the ability to place the device or the function of the implant
- GLP-1 receptor agonist therapy

- Known ischaemic heart disease or heart failure
- History of stroke
- Active Helicobacter pylori (Note: Subjects may be enrolled if they had a prior history of Helicobacter Pylori and were successfully treated)
- Actual iron deficiency anemia
- Subjects or Family history of a known diagnosis or pre-existing symptoms of systemic lupus erythematosus, scleroderma, or other autoimmune connective tissue disorder
- Known malignancy or any other multimorbid patient condition or circumstance, which, in the opinion of the investigator, would affect the patient's ability to participate in the protocol or would put the participant at an unjustified risk

# Clinical endpoints

## Primary endpoints

- Change in the glucose infusion rate (Botnia Clamp) adjusted for body weight at baseline from baseline to 4 and 36 weeks after the Endobarrier implantation, respectively
- Change in early insulin response from baseline to 4 and 36 weeks after the Endobarrier implantation, respectively

## Secondary endpoints

- Change in the glucose infusion rate (Botnia Clamp) adjusted for body weight at baseline from baseline to 24 weeks after the Endobarrier explantation
- Change in early insulin response from baseline to 24 weeks after the Endobarrier explantation, respectively
- Change in body mass index from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation
- Change in body weight from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation
- Change in HbA1c from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation
- Change in lactulose/mannitol ratio from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation
- Change in glucose AUC (MMT) from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation
- Change in c-peptide AUC (MMT) from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation
- Change in UKPDS CHD risk from baseline to 4 and 36 weeks after the Endobarrier implantation and 24 weeks after the Endobarrier explantation

# Sample Size

This study is planned as a mechanistic pilot trial to gather first data about the parameter outlined in the primary and secondary parameter section. Currently, insufficient data are available on parameters for estimating the sample size required for this pilot trial. However, previous pilot trials investigating measurements of glucose metabolism with EndoBarrier™ have used 10-16 subjects to demonstrate improvements[25, 26]. Therefore, we aim to include 10 subjects in our trial.

# Statistical Analyses

## Time of statistical analysis

All statistical analyses will be performed after the completion of trial and dataset closing.

## Analysis population

All analyses will be performed on the 'Intention to treat (ITT)' or 'full analysis set' population. All patients who will be randomized to treatment sequences will be considered for the ITT analysis.

## Data inspection

Data will be received in Microsoft Excel format and will be imported into statistical softwares for the analysis. First, data will be inspected for data entry errors on 10% of randomly selected data. Afterwards, data will be inspected for outliers and implausible values and these data points will be verified from the clinical report forms.

## Summarization of data

Qualitative variables will be summarized as frequency with corresponding percentage (%), while quantitative variables will be summarized as either mean ± standard deviation (SD) or median and interquartile range (IQR) as appropriate. Both quantitative and qualitative variables will be presented in the table.

## Statistical procedures

Normality of quantitative variables will be assessed using Shapiro-Wilk tests. The choice of statistical procedures for both primary and secondary quantitative endpoints will depend upon the distribution of variables. If normality assumption is violated, no data transformation will be carried out.

The early insulin response will be estimated from the MMT using the formula (30 minutes insulin – basal insulin)/(30 minutes glucose). The AUCs are determined by the trapezoidal method. Based on IVGTT measurements, the incremental trapezoidal area of insulin and C-

peptide during the first 10 minutes after glucose administration will represent the first-phase insulin response or acute insulin response (AIR). AIR will be also measured as the sum of insulin and C-peptide concentrations during the first 10 minutes after the glucose challenge.

Insulin sensitivity will be assessed as the mean/median glucose uptake, calculated from the glucose infusion rates during the last 60 minutes of the euglycemic clamp (GIR), which will be adjusted for baseline body weight. Changes in body weight will be analyzed as the difference in absolute total body weight, change in BMI, excess weight loss, and total body weight loss. Changes in T2DM will be analyzed as the absolute change in HbA1c, fasting plasma glucose, and changes in T2DM medication. Differences in lipid levels and blood pressure will be analyzed as absolute differences.

The repeated measures analysis of variance (ANOVA) or Friedman test will be applied to compare parameters over time and the Durbin–Conover test with Bonferroni corrections will be applied for post hoc multiple pairwise comparisons of parameters.

The weight adjusted glucose infusion rate (GIR) changes will be assessed with a multilevel non-linear mixed model with post hoc multiple comparison and Bonferroni corrections. All statistical analyses will be conducted in Stata version 17.0 and R version 4.0.

### Missing data

Missing data will be treated as such, and no data will be imputed.

### Level of significance

The two-sided alpha level of <0.05 will considered to decide statistical significance.

## Data presentation

Baseline characteristics will be tabulated for all patients. The results of primary and secondary outcomes will be presented both in tables and bar graphs, as appropriate.

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