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Statistical Analysis Plan

TRIAL FULL TITLE	Comparison of the effects of the Long Limb to the Standard Limb gastric bypass on type 2 diabetes mellitus. The LONG LIMB trial.
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Abbreviations and Definitions

AE	Adverse Event
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
BMI	Body Mass Index
BMR	Basic Metabolic Rate
LOCF	Last Observation Carried Forward
MCR	Metabolic Clearance rate of Glucose
MMTT	Mixed Meal Tolerance Test
NICE	National Institute for Health and Care Excellence
NMDS	Non-metric MultiDimensional Scaling
PANAS	Positive and Negative Affect Schedule
PERMANOVA	PERmutational Multivariate ANalysis Of Variance
Ra	Rate of glucose appearance
Rd	Rate of glucose disposal
RYGB	Roux-en-Y Gastric Bypass
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
T2DM	Type II Diabetes Mellitus
VAS	Visual Analogue Scale
WHO	World Health Organization

Introduction

The most effective and durable treatment for both obesity and type 2 diabetes mellitus (T2DM) remains bariatric surgery. Alternative surgical techniques have been sought to improve the rates of T2DM remission. Standard Limb RYGB and biliopancreatic diversion (BPD) has been showed to lead to a matched total body weight loss of 33% at 2 years post-operatively. Unfortunately, the BPD procedure has the distinct disadvantage of a substantially higher risk of developing severe nutritional complications, and this has limited its use.

To improve the glucose-lowering efficacy of Standard Limb RYGB, whilst avoiding the high risk of complications with the BPD procedure, the Long Limb RYGB has been devised as a hybrid operation that combines the standard design of Standard Limb RYGB, but with a longer biliopancreatic limb. Previous research has suggested that the rates of any complications, including nutritional, were not higher than those reported after Standard Limb RYGB.

The main anatomical difference between Long Limb RYGB and Standard Limb RYGB is that the segment of the bypassed proximal intestine, the biliopancreatic limb, is longer (150 vs. 50cm respectively). This means that in the Long Limb RYGB the common channel is shorter, and as a result nutrients reach the distal small bowel faster and in a less-digested state. The hypothesis is that the Long Limb RYGB is better for treatment of T2DM because:

a) It increases post-prandial secretion of gut hormones, and in particular glucagon-like peptide-1 (GLP-1) which results in the immediate post-prandial insulin secretion significantly higher than the Standard Limb RYGB.
b) It increases insulin sensitivity significantly more than the Standard Limb RYGB, before and after weight loss has taken place.

The trial will evaluate the efficacy on T2DM of the Long Limb RYGB compared to the Standard Limb RYGB, and investigate the mechanisms underlying any potential differences by conducting:

1. Mechanistic assessments with Mixed Meal Tolerance Test (MMTT) and Hyperinsulinaemic Euglycaemic Clamp at: pre-operative, early mechanistic post-operative (at 1-2 weeks after the surgery) and late mechanistic post-operative visits (at 20% total body weight loss after the surgery).
2. Clinical assessment pre-operatively, at the day of surgery and at 3, 6 and 12 months post-operatively.

Study Methods

General Study Design and Plan

The study is a prospective double-blinded randomised controlled parallel group clinical trial. Patients were recruited from the Imperial Weight Centre and the King's College Obesity Clinic and randomised to either the Long Limb or the Standard Limb RYGB surgery.

Inclusion-Exclusion Criteria

Inclusion Criteria

The participants must have met ALL of the following criteria to be considered eligible for the study:

- Male or female participants
- Aged between 18-70 years
- Diagnosed with T2DM according to WHO 2006 and 2011 criteria
- HbA1c ≥7.0% (≥53.0 mmol/mol) on screening
- Body mass index (BMI) ≥ 30 kg/m^2 and eligible for bariatric surgery based on NICE guidance
- On glucose-lowering medications
- Willing to comply with study requirements and able to give informed consent

Exclusion Criteria

Participants were not allowed to enter the study if ANY of the following applied:

- History of any medical, psychological or other condition, or use of any medications, including over-the-counter products, which, in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer.
- Without access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study.
- Specific contraindications to bariatric surgery
- Previous bariatric surgery
- Diagnosed with Type 1 diabetes mellitus
- Donated blood during the preceding 3 months or intention to do so before the end of the study Current pregnancy or breastfeeding
- Inability to maintain adequate contraception

Randomisation and Blinding

Participants were randomised to Long Limb or Standard Limb RYGB surgery in a 1:1 ratio. All patients were randomised in a single stratum.

Study Variables

Summary of study data and timing of measurements

The study measured patients at the following timepoints:

- Screening
- Pre-operative mechanistic visit
- Day of the operation
- Early mechanistic post-operative visit – 1-2 weeks after operation
- Late mechanistic post-operative visit – at 20% total body weight loss of the pre-operative value
- 3 months post-operatively
- 6 months post-operatively
- 12 months post-operatively

Table 1 outlines the key study measurements, and the timing of these measurements.

Table 1. Summary of study measurements

	Screening visit	Pre-op visit	Operation	Early mechanistic post-op visit	Late mechanistic post-op visit	3, 6 months post-op	12 months post-op
Demographics, duration of T2DM	x						
Weight/weight loss, BMI			x			x	x
HbA1c			x			x	x
Other blood tests			x				x
BP/heart rate	x	x		x	x		x
Body composition and measurements, BMR	x	x		x	x		x
Comorbidities and King's Score	x						x
Medications			x			x	x
Food diary		x		x	x		
Rates of T2DM remission							x
Bowel movements							x
GLP-1, GIP, PYY, bile acids, FGFs		x		x	x		
Fasting Plasma Glucose		x	x	x	x		x
Postprandial plasma glucose		x		x	x		
Markers of insulin secretion		x		x	x		
Ra, Rd, MCR from Clamp		x		x	x		
VAS		x		x	x		
Microbiota, metabolomics		x		x	x		
Faecal calories		x					x
Adverse events							x

Sample Size

The sample size was based on the primary outcome, GLP-1 concentration after the Mixed Meal Tolerance Test.

The study was powered to detect a difference in peak of active GLP-1 of 10.0 pmol/L, equating to the previously estimated change in GLP-1 post-surgery in the Standard Limb RYGB group. The standard deviation of the change in outcome values was estimated to be 10.8 pmol/L within each group. It is calculated that, with a 5% significance level and 90% power, a sample size of 20 patients in each arm was required. Based on experience, it was estimated that up to 20% of patients will drop-out of the study. To allow for this, 25 patients in each arm of the trial were planned, 50 patients in total.

Study Objectives and Endpoints

Study Objectives

The study will assess the following research questions:

The primary objective is to compare Long Limb and Standard Limb RYGB in terms of the change in peak of active GLP-1 concentration after the mixed meal tolerance test 1-2 weeks after the surgery.

The secondary objectives are to compare Long Limb and Standard Limb RYGB in terms of a number of other efficacy outcomes, and also to compare surgical methods in terms of their safety.

Demographic and Baseline measurements

The following demographic and baseline characteristics of the study participants will be collected:

- Age
- Gender
- Ethnicity
- Duration of T2DM
- Height

Endpoints

Primary outcome measure

The primary study endpoint is:

- Peak of active GLP-1 concentration after the mixed meal tolerance test 1-2 weeks after the surgery.

Secondary outcome measures

The secondary endpoints are measured at several different timepoints.

Endpoints from mixed meal test at late mechanistic post-operative visit:

- Peak of active GLP-1 concentration

Endpoints from both early and late mechanistic post-operative visits:

a) From the Hyperinsulinaemic Euglycaemic Clamp
    - Rate of glucose appearance (Ra) and disposal (Rd) and metabolic clearance rate of glucose (MCR) - basal, low and high
b) From the MMTT:
    - Active GLP-1 concentration (AUC)
    - Total GLP-1 (peak, AUC)
    - PYY (peak, AUC)
    - GIP (peak, AUC)
    - Markers of insulin secretion (peak, AUC)
    - Plasma glucose (peak, AUC)
    - Bile acids (peak, AUC)
    - FGF-19 and 21 (peak, AUC)
    - Systolic and diastolic blood pressure (AUC)
    - Heart rate (AUC)
    - Appetite ratings (Visual Analogue Scales; AUC)
c) Blood, urine and faecal microbial diversity and metabolomics
d) Total caloric intake and macronutrient composition - % fat, % protein, % carbohydrates
e) Body composition and Basic Metabolic Rate (BMR)

Endpoints at the day of surgery:

- Common channel length
- Total small bowel length
- Proportion of common channel to total small bowel length
- Operating time
- Length of in hospital stay

Endpoints at 3, 6 and 12 months:

- HbA1c
- % of total body weight loss
- Number of glucose lowering medications

Secondary endpoints at 12 months:

- % of total body weight loss
- BMI
- Body composition
- Basic Metabolic Rate (BMR)
- Waist, hips and neck measurements
- T2DM remission
- Comorbidities
- Medications
- King's Obesity Staging Score
- Systolic and diastolic blood pressure

- Heart rate
- Oxygen saturation
- Bowel movements frequency
- Blood tests: fasting plasma lipids concentration, fasting plasma glucose, haematinics, vitamins
- Total caloric intake
- Macronutrient composition - % fat, % protein, % carbohydrates
- Faecal caloric content

Safety outcomes

Safety will be assessed by the recording of adverse events (AEs) and serious adverse events (SAEs) reported during the operation for up to one-year following surgery. This will include medical, surgical, nutritional and psychological complications adverse events.

An SAE will be defined as an adverse event that meets any of the follow criteria:

- Leading to death
- Life-threatening
- Leads to hospitalisation or prolongation of existing hospitalisation
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect.

Derived values

Peak

For each outcome, the peak will be defined as the maximum post-meal concentration (i.e. from time 15 onwards) per patient, regardless of at which timepoint the peak concentration was achieved.

Area Under the curve (AUC)

A number of outcomes from the mixed-meal tests will be summarised by the Area under the curve values. The AUC will be calculated using the trapezium rule.

For outcomes with measurements at time -30, the first value used in the calculation will be the mean of the -30 and 0 timepoints. When there is no -30 value, the time 0 value will be used as the first measurement in the calculation.

Absolute changes from baseline

Absolute changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the outcome timepoint.

Percentage changes from baseline

Percentage changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the outcome timepoint, dividing this sum by the baseline value and multiplying by 100.

General Considerations

Timing of Analyses

A single analysis will take place at the completion of the study, after all data is collected. No interim analyses will be performed.

Analysis Populations

Full Analysis Population

The Full Analysis Population will consist of patients in the groups to which they were randomised, regardless of deviation from the protocol or whether they received the allocated surgery. Patients with completely missing data at the outcome timepoint will be excluded from this dataset for the particular outcome for which they had missing data.

Per Protocol Population

The Per Protocol patient population will consist of those patients who received the surgery that they were randomised to. Patients receiving surgery different to their allocation will be excluded from this population. Analyses will only be performed using this population if it differs from the Full Analysis Population.

Safety Population

The safety population will consist of all patients recruited into the study who participate for at least one week of the study. This dataset will analyse patients in the groups to which they were randomised, regardless of deviation from the protocol or whether they received the allocated surgery.

Subgroups

All patients will be analysed together, with no subgroup analyses performed.

Missing Data

At any timepoint, if there is no data at all for a given outcome, patients with missing data will be excluded from the analysis, and only observed data will be analysed. Missing data will be assumed to

be Missing At Random. No imputation procedures will be employed to deal with missing data if it is completely missing at a given timepoint.

During the MTTT, data is collected serially over a short time period. For patients with some data collected, but missing information from the final serial measurements, a Last Observation Carried Forward (LOCF) approach will be used to impute missing data.

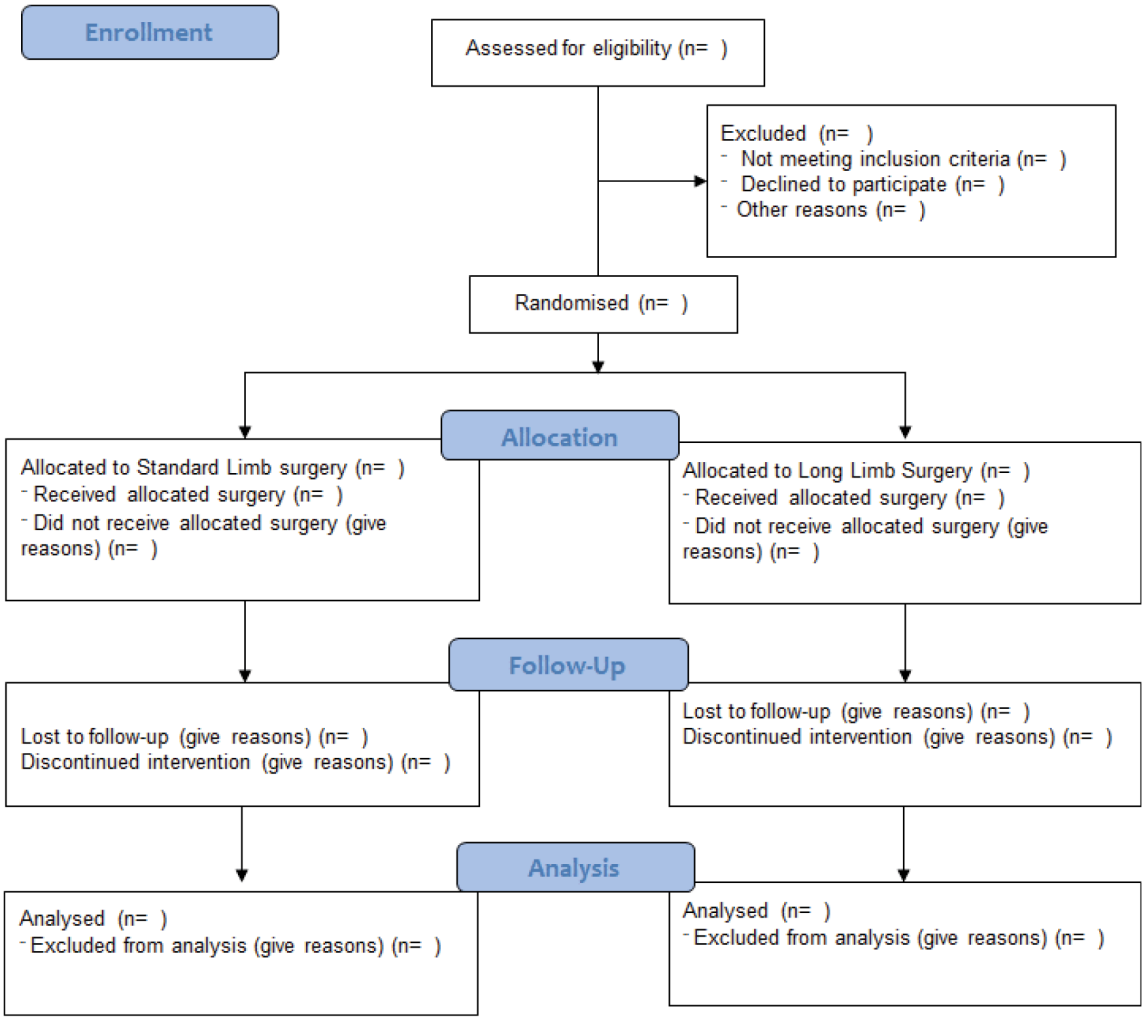
Summary of Study Data

Subject Disposition

A summary of the number of subjects that reached the various stages of the study will be summarised. Reasons for non-participation and withdrawal will be summarised.

A CONSORT diagram will be produced, such as Figure 1, which will illustrate the flow of patients throughout the study.

Figure 1. Outline CONSORT diagram



Descriptive Analysis Methods

Continuous variables will be summarised using the number of (non-missing) datapoints, mean and standard deviation if found to follow a normal distribution. Continuous variables not found to be normally distributed will be summarised by the number of datapoints, median and inter-quartile range. Categorical variables will be summarised by the frequency and percentage (based on the non-missing sample size) of values in each category.

Demographic and Baseline Variables

Baseline values for each parameter are defined as the last value measured before the intervention, i.e. surgery. A summary of patient demographics and baseline measurements are outline in Section 5.4.2.

Descriptive statistics will be produced for these variables for each of the two study arms separately.

The summary statistics will be produced in accordance with section 8.2. No hypothesis tests will be performed to compare the two groups at baseline.

Efficacy Analyses

Primary Efficacy Analysis

The primary endpoint of the study is the peak of active GLP-1 concentration at the early mechanistic post-op visit.

The analysis will be performed using Analysis of Covariance (ANCOVA). In the analysis, the peak of active GLP-1 concentration at the early mechanistic post-operative visit will be considered as the outcome measure, whilst baseline peak of active GLP-1 will be included as a covariate.

If the data does not meet the assumptions of the ANCOVA analysis (e.g. normally distributed residuals, homogeneity of variance), a data transformation of the outcome variable (e.g. log transformation) before analysis will be utilised to ensure that the assumptions are met.

The summary statistics for the outcome variable will be produced in accordance with section 8.2. The baseline adjusted difference in outcome values between groups will be reported, along with a corresponding 95% confidence interval.

The primary study analysis will be performed using the Full Analysis Population (see section 7.2.1).

Secondary Efficacy Analysis

Secondary outcomes measured on a continuous scale, with a baseline measurement, will be analysed using a similar approach to that outline for the primary efficacy outcome. The data from each post-operative timepoint will be analysed in a separate analysis.

For continuous secondary outcomes where there no baseline measurement, the two groups will be compared using the unpaired t-test. Alternatively, the Mann-Whitney test will be used if the assumptions of the t-test are not met.

For the continuous outcomes, the exceptions to the previously described methods are for the blood, urine and faecal microbial diversity and metabolomics outcomes. These will be analysed using non-metric multidimensional scaling (NMDS) plots. Additionally, PERMANOVA p-values will be generated using the UniFrac weighted distance matrix generated from Mothur. Family-level extended error bar plots will be generated, White's non-parametric t-test with Benjamini-Hochberg FDR will be utilised.

Binary and nominal outcomes (e.g. achieving diabetes remission) will be compared between the two study groups using either the Chi-square test, or Fisher's exact test if the number of responses in some categories is low.

Ordinal outcomes will be analysed using the Mann-Whitney test to allow for the natural ordering of the response categories.

The secondary efficacy analyses will be performed using the Full Analysis Population.

Sensitivity Efficacy Analysis

If the Per Protocol Population differs in its membership from the Full Analysis Population, the primary outcome will be additionally analysed using this population. The analysis methods will be equivalent to those described in Section 9.1.

If there is no difference between the Per Protocol Population and Full Analysis Population, no sensitivity analyses will be performed.

Other Analyses

An additional set of analyses will examine the association between the key primary and secondary efficacy outcomes measured on a continuous scale and the proportion of the common channel length to the total small bowel length. The analysis will be performed using Pearson correlation. Alternatively, Spearman's rank correlation will be used if the Pearson correlation assumptions (e.g. non-linear relationship, both variables non-normally distributed) are not met. For each outcome, a single analysis will be performed for all patients, combining the two study arms together.

Safety Analyses

The main safety outcome is the occurrence of adverse events (AEs). For each of the study groups, the number of adverse events in each group and per patient will be summarised. Specific details of the adverse events will be recorded in addition to the number of AEs that are serious and non-serious.

If it is deemed that there are sufficient occurrences of adverse events in total, a formal test of significance will be performed to compare AE occurrence at the patient level between study arms. Fisher's exact test will be used for this analysis. However, it is acknowledged that the study is unlikely to be powered to show a difference between groups for this endpoint.

Technical Details

The data analysis will be primary performed using the statistical software packages Stata (version 15.1), SPSS (version 20 or later), GraphPad PRISM (version 6 or later). Programs recording details of all data manipulation and data analyses will be produced and kept, so that the analyses can be externally inspected and, if necessary, re-run.

The exception is for the analysis of the blood, urine and faecal microbial diversity and metabolomics outcomes, which will be analysed using the Vegan library within the R statistical package and the Statistical Analysis of Metagenomic Profiles software package.

Summary of Changes to the Analysis Plan

Changes from Version 1.0 to 1.1

The following changes were made between the SAP versions:

- Reformatting of the order the sections
- Omission of demographic summaries for both study groups combined
- Addition of further secondary outcomes
- Details of LOCF approach to be used for MMTT data

Changes from Version 1.1 to 1.2

The following changes were made between the SAP versions:

- Change of approach for secondary outcomes from the MMTT to consider all measurements in the analysis, and not summary measures (peak, AUC).
- Change of statistical methods for secondary analysis of MMTT outcomes to reflect the change of approach for these outcomes

Changes from Version 1.2 to 1.3

The following changes were made between the SAP versions:

- Change of approach for secondary outcomes from the MMTT to consider analysing as summary measures (peak, AUC) rather than using all individual measurements in the outcomes.
- Change of statistical methods for secondary analysis of MMTT outcomes to reflect the change of approach for these outcomes

Further changes to the SAP

If there are further revisions to the original proposed analyses, or if any supplementary analyses are planned, these will be documented in a future version of the SAP. The reason for any changes/additions will be documented.