LPBSP Study Protocol

Longitudinal Phenotyping of Bariatric Surgery Patients

Version 1.5 (16/02/2019)

MAIN SPONSOR: Imperial College London
FUNDERS: Imperial College London
STUDY COORDINATION CENTRE: St Mary's Hospital
NRES reference: 15/ES/0026
Protocol number: 15SM2479
IRAS project ID: 169767
Tissue Bank registration: Gas-AD-15-020

Protocol authorised by:

Name & Role	Date	Signature

Study Management Group

Chief Investigator: Nicholas Penney
Principal Investigator: Professor A Darzi

Co-investigators: Mr S Purkayastha, Professor E Holmes

Study Management: Nicholas Penney

Study Coordination

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: Nicholas Penney

Address: 10th Floor QEQM, St Mary's Hospital, Imperial College Healthcare NHS Trust, London W2 1NY
Tel: 07545461256
E-mail: n.penney@imperial.ac.uk

Clinical Queries

Clinical queries should be directed to Mr S Purkayastha – Consultant Bariatric Surgeon, who will direct the query to the appropriate person. (purkayastha.purkayastha@nhs.net)

Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office
Imperial College London & Imperial College Healthcare NHS Trust
5th Floor, Lab Block
Charing Cross Hospital
Fulham Palace Road
London
W6 8RF
Tel: 0203 311 0212
Fax: 0203 311 0203

Funder

Imperial College London & the Diabetes Research and Wellness Foundation

This protocol describes the 'Phenotyping Bariatric Surgery Patients' study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Table of Contents

1. INTRODUCTION 6

1.1 BACKGROUND 6

2. STUDY OBJECTIVES 7

3. STUDY DESIGN 7

3.1 STUDY OUTCOME MEASURES 7

4. PARTICIPANT ENTRY 8

4.1 PRE-REGISTRATION EVALUATIONS 8

4.2 INCLUSION CRITERIA 8

4.3 EXCLUSION CRITERIA 8

4.4 WITHDRAWAL CRITERIA 8

5. ADVERSE EVENTS 8

5.1 DEFINITIONS 8

5.3 REPORTING PROCEDURES 8

6. ASSESSMENT AND FOLLOW-UP 9

7. STATISTICS AND DATA ANALYSIS 10

8. REGULATORY ISSUES 10

8.1 ETHICS APPROVAL 10

8.2 CONSENT 10

8.3 CONFIDENTIALITY 10

8.4 INDEMNITY 11

8.5 SPONSOR 11

8.6 FUNDING 11

8.7 AUDITS AND INSPECTIONS 11

9. STUDY MANAGEMENT 11

11. PUBLICATION POLICY 11

12. REFERENCES 11

GLOSSARY OF ABBREVIATIONS

T2DM	Type 2 Diabetes Mellitus
LRYGB	Laparoscopic Roux-en-Y Gastric Bypass
LSG	Laparoscopic Sleeve Gastrectomy
mRYGB	Mini Roux-en-Y Gastric Bypass
LAGB	Laparoscopic Adjustable Gastric Band

KEYWORDS
Obesity, Bariatric surgery, Type 2 diabetes mellitus, Gut microbiome, Metabonome

STUDY SUMMARY

TITLE	Phenotyping of Bariatric Surgery Patients
DESIGN	Observational study of obese patients undergoing bariatric surgery
AIMS	To identify novel preoperative biomarkers of diabetes resolution following bariatric surgery
OUTCOME MEASURES	HbA1c, Weight / BMI
POPULATION	Obese (BMI>30kg/m^2) patients undergoing bariatric surgery
ELIGIBILITY	See Inclusion / Exclusion criteria
DURATION	18-month recruitment period. 2 year follow up

1. INTRODUCTION

1.1 BACKGROUND

Within the UK approximately 1 in 4 adults are now obese. This obesity epidemic is having a dramatic impact on the rise of associated chronic disease states such as type 2 diabetes mellitus (T2DM), cardiovascular disease and cancer, which in turn carry a significant global health and economic burden. The Department of Health estimates the financial cost to the NHS at £5 billion annually. Thus there is an urgent need for effective preventative and therapeutic strategies for the treatment of obesity. Disappointingly, the encouragement of lifestyle changes such as improved diet and increased exercise together with pharmacological therapies has proved largely ineffectual thus far. Hence surgical procedures have been developed to achieve more sustainable weight loss and are currently the gold standard treatment for morbid obesity. The procedures were originally designed to cause weight loss through gastric restriction and / or malabsorption. However, in addition to weight loss they have also been highly successful in the resolution of co-morbidities including diabetes, heart disease, sleep apnoea and in reducing cancer risk[1]. Due to these metabolic effects they are termed 'metabolic' procedures. The metabolic outcomes are achieved through both weight-dependent and interestingly, weight-independent mechanisms[1, 2]. Indeed, many of the metabolic effects are seen before weight loss has occurred. This is because bariatric surgery induces a complex systems wide metabolic effect including disruption of the gut microbiome-host metabolic axes.

The gut microbiome has now been linked extensively with numerous disease states and it represents a novel therapeutic target in the treatment of obesity[3]. A low bacterial richness in the gut is associated with increased adiposity, insulin resistance and dyslipidaemia[4]. Indeed metagenome wide association studies have demonstrated the ability to identify T2DM patients based on their gut microbiome[5]. Convincingly, in an effort to establish causality, the colonisation of germ free mice with the faecal microbiota of twin pairs discordant for obesity results in significantly greater increases in body mass and adiposity in mice receiving the faecal microbiota from the obese twin[6]. However, the overwhelming disruption to the gut microbiome caused by bariatric surgery is only just being defined, as is its functional importance. This has been demonstrated in faecal transplant experiments using the gut microbiota from Roux-en-Y gastric bypass (RYGB) treated mice to transfect non-operated germ free mice, leading to significant weight loss and decreased fat mass compared to transfer of the gut microbiota from mice who had undergone sham surgery[7]. This is thought to be through mechanisms including: reduced energy harvest of non-digestible food types such complex carbohydrates and plant polysaccharides, increased gut permeability leading to increased systemic inflammation, altered bile acid metabolism and carbohydrate fermentation products such as short-chain fatty acids acting as signalling molecules to increase satiety[8, 9]. In particular BAs have emerged as key signalling molecules, acting through the Farnesoid X Factor nuclear receptor (FXR) and G protein-coupled bile acid receptor (TGR5) to induce a number of physiological changes including; increased incretin release, improved glucose homeostasis, improved satiety, altered gut microbiota – acting in a bi-directional way, and increased metabolic rate[10, 11]. In support of this, many of the beneficial effects seen after RYGB are reproduced in rats by simply diverting bile from the common bile duct to the mid to distal jejunum via a catheter[10]. Additionally, following Vertical Sleeve Gastrectomy (VSG) in mice, body fat is reduced and glucose tolerance improved compared to sham-operated controls. However, body fat and glucose tolerance do not significantly differ in FXR knockout mice after VSG. Furthermore, *Bacteroides* were reduced following VSG in the wild type mice but the gut microbiota was not altered in the FXR knockout mice suggesting that FXR signalling is important in mediating the beneficial effects of VSG, in part through its modulation of the gut microbiota[11].

1.2 RATIONALE FOR CURRENT STUDY

From a clinical perspective, national and international guidelines for bariatric surgery have historically been based predominantly on weight. However, bariatric surgery is also highly successful in achieving remission of co-morbidities such as T2DM. The chance of remission does not appear to be weight dependent. In a recent meta-analysis T2DM remission was 72% in patients with a BMI<35kg/m^2 and 71% in patients with a BMI>35kg/m^2 undergoing bariatric surgery[12]. Consequently, the latest guidelines by NICE in the UK have been updated to reflect the convincing body of evidence that surgery is also a highly successful and cost-effective treatment for T2DM patients who are less obese. As many as 2 million patients may be eligible for bariatric surgery under these new guidelines. However, unfortunately up to 30% of T2DM patients who undergo bariatric surgery do not achieve remission of their T2DM. There is currently no reliable method of identifying these patients preoperatively. T2DM remission does not appear to correlate with preoperative BMI or any other known clinical or biochemical marker reliably. Therefore novel preoperative biomarkers to help guide clinicians and policy makers select the ideal surgical candidates are urgently required to enable truly personalised healthcare.

2. STUDY OBJECTIVES

We hypothesise that weight loss and the metabolic effects of bariatric surgery are achieved in part through a functional modification of the gut microbiome and altered microbiome – host metabonome interactions. We aim to elucidate these and other mechanisms of T2DM remission following bariatric surgery. We will explore both the weight dependent and weight independent mechanisms with the translational objectives of 1) identifying novel, surgical and non-surgical, therapeutic targets and 2) identifying novel biomarkers for preoperative prognostication of T2DM remission.

3. STUDY DESIGN

Type of study: Longitudinal phenotyping of bariatric surgery patients with collection of tissue samples (blood, urine, faeces + *resected stomach from VSG patients*) and clinical data pre and postoperatively.

Duration: Patients will be followed up 2 years postoperatively. Total study time including recruitment = 60 months.

Patients: Obese patients undergoing bariatric surgery (RYGB / VSG / mRYGB / LAGB).

3.1 STUDY OUTCOME MEASURES

The 1^0 clinical outcome measure will be diabetes remission defined as HbA1c <42mmol/mol. The 2^0 clinical outcomes will be partial diabetes remission (HbA1c <48mmol/mol), HbA1c reduction and % excess weight loss.

These clinical outcomes will be mapped to metabonomic and metagenomic data to identify preoperative markers of diabetes remission and weight loss.

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

Potential participants will have their notes screened by NHS team members to identify patients that meet the inclusion / exclusion criteria. They will then be asked if they are happy to talk to the research team to discuss the project and undergo informed consent to take part.

4.2 INCLUSION CRITERIA

Referred to the Imperial Weight Centre (NHS) Tier 4 service team for consideration bariatric surgery (RYGB / VSG / mRYGB / LAGB), Obese (BMI>30kg/m^2), Age ≥18, failure of efforts at lifestyle modification and dieting, fitness for anaesthesia and procedure, willingness to comply with the trial protocol.

4.3 EXCLUSION CRITERIA

Previous bariatric surgery, previous major abdominal surgery*, pregnancy or intention to become pregnant during trial period, lack of capacity to consent.

* = Small or large bowel resection, liver, pancreatic or splenic surgery, as these will influence the bacteria in the gut and / or the patient's metabolic state, which we are studying. It will not include patients that have previously had an appendicectomy, cholecystectomy, hernia repairs or surgery on other organs not listed above.

4.4 WITHDRAWAL CRITERIA

Grade IV or higher surgical complication (Modified Clavien classification).
Patient choice for any reason.

5. ADVERSE EVENTS

As no added clinical interventions / procedures will occur in this study NO adverse events are expected as a result of participating.

5.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs
All such events, whether expected or not, should be recorded.

5.3.2 Serious AEs
An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the East of Scotland Research Ethics Service where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
Email: eosres.tayside@nhs.net
Please send SAE forms to: TAyside medical Science Centre Residency Block Level 3, George Pirie Way, Ninewells Hospital and Medical School Dundee DD1 9SY
Tel: 01382 383871 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

Patients will undergo follow up according to the usual bariatric surgery follow up protocol at the Imperial Weight Centre.

- At patients usual preoperative, 3 month, 1 and 2 year postoperative outpatient appointments patients will provide additional samples of blood, urine and faeces for analysis.
- Patients will be given brief online 24-hour diet recall questionnaires to complete while waiting for their usual outpatient appointments. They will then be asked to complete entries for two days following each hospital visit using a personal computer via a personal link to a secure website provided.
- Patients will be sent home with 24-hour urine collection bottles. They will be asked to provide 24-hour collections for before surgery and the days before their 3-month and 1-year outpatient visits. They will be able to return their samples when they attend these appointments.
- At the time of surgery researchers will collect resected stomach tissue from LSG patients that would usually be disposed.
- Clinical data will be retrieved from the medical notes preoperatively to 2 years postoperatively.

Study End: 130 type 2 diabetic and 65 non-diabetic patients will be recruited and followed-up until 2 years postoperatively.

7. STATISTICS AND DATA ANALYSIS

Multivariate statistical analysis will be performed on the generated metabonomic and metagenomic data sets. This will include unsupervised principal components analysis (PCA) and supervised partial least squares discriminant analysis with orthogonal signal correction (OPLS-DA) and O2-PLS between data sets. Mapping T2DM outcomes to metabonomic and metagenomic data will potentially identify novel preoperative biomarkers that are able to prognosticate T2DM resolution following bariatric surgery.

Due to the complexity of metabonomic data sample size calculations are difficult and there is currently no standardised technique. We used peer-reviewed MetSizeR methodology to calculate appropriate sample sizes[13]. Using principal component analysis on an expected metabonomic data set of 300 metabolites of which 20% differentiate significantly and a targeted false discovery rate of 5%, over 15 samples are needed in each group. Approximately 30% of obese diabetic patients do not have resolution of disease following bariatric surgery[12]. To allow for an expected drop out rate of 20% at 1 year we will recruit 65 patients undergoing RYGB and 65 patients undergoing VSG to achieve 15 patients with non-resolution of diabetes and 36 patients with resolution in each surgical group. We will also aim to recruit 65 non-diabetic patients undergoing surgery to act as a control group. This will enable comparison against diabetic patients that undergo remission after surgery.

Four surgeons at the Imperial Weight Centre collectively perform approximately 220 RYGB and 180 VSG procedures a year. In previous years 25% of patients have had T2DM. However, we expect this percentage to rise since the introduction of new NICE guidelines encouraging bariatric surgery in diabetic patients in November 2014. We expect to recruit 1-2 patients a week that meet the inclusion criteria and have completed recruitment after 18 months.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Chief Investigator has obtained approval from the East of Scotland Research Ethics Committee. The study will be submitted for a Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

The study is funded by the Diabetes Research and Wellness Foundation.

8.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Nicholas Penney.

10. PUBLICATION POLICY

The trial will be registered on ClinicalTrials.gov before the start of recruitment.
The anonymous research data will be published in a scientific journal. This will be open-access.
Deidentified research data from this project will be deposited in public data repositories to ensure that the research community has long-term access to the data and that the research data is migrated to new formats, platforms, and storage media.

10. REFERENCES

1. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD *et al.* Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. *N. Engl. J. Med.* 2014; **370**(21)**:** 2002-13.

2. Cummings BP, Bettaieb A, Graham JL, Stanhope KL, Kowala M, Haj FG *et al.* Vertical sleeve gastrectomy improves glucose and lipid metabolism and delays diabetes onset in UCD-T2DM rats. *Endocrinology* 2012; **153**(8)**:** 3620-32.

3. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**(7122)**:** 1027-31.

4. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**(7464)**:** 541-6.

5. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**(7418)**:** 55-60.

6. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL *et al.* Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; **341**(6150)**:** 1241214.

7. Liou AP, Paziuk M, Luevano JM, Jr., Machineni S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci. Transl. Med.* 2013; **5**(178)**:** 178ra41.

8. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489**(7415)**:** 242-9.

9. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE *et al.* A core gut microbiome in obese and lean twins. *Nature* 2009; **457**(7228)**:** 480-4.

10. Kohli R, Setchell KD, Kirby M, Myronovych A, Ryan KK, Ibrahim SH *et al.* A surgical model in male obese rats uncovers protective effects of bile acids post-bariatric surgery. *Endocrinology* 2013; **154**(7)**:** 2341-51.

11. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R *et al.* FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014; **509**(7499)**:** 183-8.

12. Panunzi S, De Gaetano A, Carnicelli A, Mingrone G. Predictors of Remission of Diabetes Mellitus in Severely Obese Individuals Undergoing Bariatric Surgery: Do BMI or Procedure Choice Matter? A Meta-Analysis. *Ann. Surg.* 2014.

13. Nyamundanda G, Gormley IC, Fan Y, Gallagher WM, Brennan L. MetSizeR: selecting the optimal sample size for metabolomic studies using an analysis based approach. *BMC Bioinformatics* 2013; **14:** 338.