# Bile Reflux Post-Bariatric Surgery: A Cohort Study

## STUDY PROTOCOL FOR SUBMISSION TO HUMAN RESEARCH AND ETHICS COMMITTEE

Version 8

Date: December 30th 2019

# 1. General Information

### 1.1. STUDY TITLE

Bile Reflux Post Bariatric Surgery: A Cohort Study

### 1.2. TRIAL REGISTRATION NUMBERS

WHO Universal Trial Number: U1111-1213-1261

Australian New Zealand Clinical Trials Registry (ANZCTR): ACTRN12618000806268

- Available at: http://www.ANZCTR.org.au/ACTRN12618000806268.aspx

### 1.3. PRINCIPAL INVESTIGATORS

**Mr. Jon Shenfine** MBBS, FRCS, FRACS, PhD
Consultant Oeophago-gastric Surgeon
Department of Surgery
Royal Adelaide Hospital
Port Road, Adelaide 5000
jon.shenfine@adelaide.edu.au

**Associate Professor George Kiroff** MBBS, MS, FRACS
Consultant Upper Gastrointestinal Surgeon
Department of Surgery
The Queen Elizabeth Hospital
28 Woodville Road, Woodville South 5011
George.kiroff@sa.gov.au

**Associate Professor Dylan Bartholomeusz** MBBS, FRACP, MD
Senior Nuclear Medicine Physician and Gastroenterologist.
Director, Department of Nuclear Medicine, PET and Bone densitometry
Royal Adelaide Hospital and SA Medical Imaging
Port Road, Adelaide 5000
Dylan.Bartholomeusz@sa.gov.au

### 1.4. ASSOCIATE INVESTIGATORS

**Dr. Thomas Eldredge** MBBS
PhD Candidate
School of Medicine, Discipline of Surgery
University of Adelaide
North Terrace, Adelaide 5000
(m) 0439 769 900
tom.andrew.eldredge@gmail.com

**Dr. Jennifer Myers** BSc, PhD
Clinical Scientist
Department of Surgery
The Queen Elizabeth Hospital and Royal Adelaide Hospital
28 Woodville Road, Woodville South 5011 and Port Road, Adelaide 5000
Jenny.myers@adelaide.edu.au

**Ms. Madison Bills** BMedRadiation in Nuclear Medicine (Hons)
Nuclear Medicine Technologist
Department of Nuclear Medicine, PET and Bone densitometry
Royal Adelaide Hospital and SA Medical Imaging
Port Road, Adelaide 5000
Madison.Bills@sa.gov.au

**Dr. Mark Harris** BMedSc, MB, BCh, BAO (Hons), BA (Hons)
Surgical Registrar
Department of Surgery
The Queen Elizabeth Hospital
28 Woodville Road, Woodville South 5011
Mark.harris2@sa.gov.au

**Dr. Matthew Watson** MBBS
Surgical Registrar
Department of Surgery
The Queen Elizabeth Hospital
28 Woodville Road, Woodville South 5011
Matthew.watson@sa.gov.au

# 2. Study Objectives

**2.1. PURPOSE OF STUDY**

**2.2.** PRIMARY OBJECTIVE

- To evaluate whether laparoscopic single anastomosis gastric bypass (LSAGB), laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic sleeve gastrectomy (LSG) operations result in increased bile reflux, as determined by the presence of bile acids in the stomach and oesophagus, with or without stomach/oesophageal tissue damage

**2.3.** SECONDARY OBJECTIVES

- To determine the impact of cholecystectomy on the rates and severity of bile reflux after weight-loss surgery
- To further evaluate the safety and efficacy of LSAGB, LSG and LRYGB
- To evaluate the patient tolerability of hepatobiliary scintigraphy, oesophageal reflux testing and endoscopy

**2.4.** RESEARCH QUESTIONS

- Do LSAGB, LRYGB and LSG increase the incidence or amount of bile refluxing into the stomach and oesophagus?
- What is the patient tolerability of hepatobiliary scintigraphy and oesophageal impedance testing?

# 3. Rationale and Background

An increasing number of patients are receiving operations for morbid obesity. Obesity is an independent risk factor for development of gastroesophageal reflux and weight-loss can significantly improve symptoms. Some weight-loss operations, however, can promote reflux due to the anatomical alterations, and reflux can persist despite weight loss (1). Duodenal contents - bile, bicarbonate and pancreatic enzymes - may reflux back into the stomach and oesophagus, termed *duodenogastrooesophageal reflux (DGOR)* or *bile reflux.* It has been well-documented that bile acids, in conjunction with gastric acid, contribute to the development of reflux-type symptoms (heartburn, regurgitation, waterbrash, etc.), erosive oesophagitis and also to the development of Barrett's oesophagus (2, 3).

### 3.1. Bile acids cause oesophageal adenocarcinoma

Bile acids form a group of amphipathic molecules synthesized in pericentral hepatocytes of hepatic acini during carbohydrate metabolism. They are steroid acids, comprising of one cyclopentane and 3 cyclohexane rings, a carboxylic side chain and varying numbers of hydroxyl groups. Under physiologic conditions, essentially all bile acids secreted into bile are conjugated (>98%) with either taurine or glycine, which lowers the acidity index and in turn increases solubility and ionization capability of bile acids. The physiological consequence of conjugation is decreased passive diffusion across cell membranes, thereby maintaining high intraluminal concentrations to facilitate their primary function of lipid digestion and absorption.

Bile acids are most injurious to oesophageal epithelial cells when they are un-ionised, hydrophobic and soluble, resulting in maximal diffusion capability across cell membranes. pKa is a key factor in bile salt solubility. Unconjugated bile acids have a pKa of around 5.1, whereas taurine and glycine conjugated acids have values of approximately pKa 4 and pKa 2 respectively. It follows that in neutral and alkaline solutions, bile acids exist predominantly in their non-injurious ionized form. Furthermore, when bile is acidified to below pH 2, irreversible precipitation of bile acids occurs, decreasing the total number of bile acids found in bile (4). Thus, in solutions with pH 2-6, typical gastric acidity level, bile acids are most injurious with a significant proportion unionized in solution. Human and animal studies support this biochemical data, showing that bile acid-mediated oesophageal damage is potentiated in an acidic compared with weakly acidic and neutral environments (5-7). The majority of duodenogastrooesophageal reflux events are known to be acidic rather than neutral or alkaline, increasing the risk of oesophageal damage (8).

Increased oesophageal epithelial permeability is a key feature of reflux-induced tissue damage in the oesophagus. Studies of animal and human oesophageal tissue, both native and cultured, show that epithelial permeability is increased by exposure to bile acids in acidic solutions, and to a lesser degree in weakly acidic and neutral solutions (5-7). Kiroff et al. evaluated this *in vivo*, in anaesthetised rabbits by measuring the rate of $H^+$ disappearance during intra-oesophageal perfusions of varying concentrations of sodium taurocholate in normal saline or 10mmol/L $H^+$ (5). Farre et al. studied rabbit oesophageal tissue *in vitro*, also following exposure to bile acids at varying concentrations and pH, and measuring transepithelial permeability by electron microscopy (6). Results were consistent in both experiments, demonstrating significantly increased $H^+$ disappearance and transepithelial permeability respectively in acidic bile acid solutions, but not in neutral bile acid solutions. Human studies have drawn similar conclusions (7, 9).

Increased epithelial permeability combined with optimal refluxate pH for diffusion of soluble, un-ionised bile acids results in increased translocation of bile acids into epithelial cells. Once intracellular, bile acids are known to incite an inflammatory response and lead to the production of reactive oxygen species, causing oxidative DNA damage and cell death (10). Through these mechanisms, tissue may progressively undergo metaplastic change. This can lead to Barrett's oesophagus and less often, oesophageal adenocarcinoma.

### 3.2. Bariatric surgery can cause bile reflux

The obesity epidemic is escalating - In 2014, more than 1.9 billion adults aged 18 and above (39% of global population) were overweight with 600 million being obese (11). Medical treatment of obesity continues to yield disappointing results. Obesity surgery consistently results in effective and sustained weight loss with improved quality of life, and remission of obesity-related comorbidities (12). Obesity is an independent risk factor for development of gastroesophageal reflux and weight-loss can significantly improve symptoms. Some weight-loss operations, however, can promote reflux due to the anatomical alterations, and reflux can persist despite the weight loss (1).

Recent advances in obesity surgery include the introduction of new surgical techniques. Currently, the laparoscopic Roux-en-Y gastric bypass (LRYGP) is the 'gold standard' and most commonly performed surgical procedure for weight loss worldwide, however it remains a technically challenging laparoscopic operation with significant associated risk. It is the gold standard as it facilitates sustained weight loss with relatively few adverse outcomes. Laparoscopic sleeve gastrectomy (LSG) is an alternative operative technique increasingly being used for weight loss, however it is associated with an increase in prevalence of gastro-oesophageal reflux. This was shown in a study of 66 obese patients evaluated 1 year after LSG and demonstrated a significant increase in endoscopy-proven gastro-oesophageal reflux and erosive oesophagitis (13).

The third alternative operative technique is the laparoscopic single anastomosis gastric bypass (LSAGB), also known as the 'mini gastric bypass' or the 'omega loop gastric bypass'. This technique is growing in popularity since being first described in 2001 (14). As described by proponents of the technique, there are many advantages: it is quicker to learn; has a shorter operative time (and thus shorter anaesthetic); reduces risk of complications (one anastomosis rather than two) and weight loss as well as glucose tolerance outcomes are comparable to LRYGP (15, 16). Despite these results, LSAGB is not being as rapidly adopted by bariatric surgeons as might be expected. The low uptake relates to controversy concerning the theoretical long-term harm and potentially carcinogenic effects of bile reflux into the gastric remnant and the oesophagus after LSAGB (17).

The effect of gallbladder removal on the incidence of bile reflux remains debatable. Increased rates and severity of bile reflux post-cholecystectomy has been reported by some studies(18, 19), and refuted by others (20). In patients undergoing weight-loss surgery, an operation that may exacerbate bile reflux, the additive impact of cholecystectomy on bile reflux remains unknown. Given the significant end-sequelae, namely carcinogenesis, the effect of cholecystectomy on bile reflux needs to be examined to guide surgical decision making.

### 3.3. Investigative techniques for diagnosis of bile reflux

The intermittent nature of DGER is one of the challenges for the development of an optimal diagnostic investigation. The available techniques include HIDA scintigraphy, endoscopy and biopsies, 'Bilitec' spectrophotometer probe and oesophageal impedance-pH testing. Of these techniques, HIDA scintigraphy is the least invasive but only provides a short window (1-2 hours) for the capture of DGER events. A more complete DGER profile requires prolonged monitoring, but none of the current ambulatory techniques are ideal. Ambulatory Bilitec monitoring was specifically developed for the detection of bile reflux, but is prone to errors, particularly false positive readings, whereas pH testing (acid reflux) and also combined impedance-pH monitoring (acid and non-acid reflux) do not directly detect bile reflux.

HIDA scintigraphy, a radio-nuclide scan, has superior patient tolerability over catheter-based methods, with a shorter monitoring time (1-2 hours compared with 24 hours) and a non-invasive nature. Published figures for sensitivity and specificity for each technique are lacking, limiting direct comparison of techniques. Of available studies, HIDA scintigraphy shows superior sensitivity over EGD and aspiration for diagnosis of DGER (21). However, for quantification of refluxate composition or for when visualization of the oesophagus is required to determine any mucosal damage then endoscopy with fluid aspiration and tissue biopsy would be most appropriate.

### 3.4. Tolerability of investigative examinations

No data exist on patient tolerability/acceptability of HIDA scintigraphy or Bilitec monitoring. Studies of other catheter-based investigations involving probes with half the diameter of a Bilitec probe, have reported a significant impact on patient comfort (22, 23). Radiation exposure during HIDA scintigraphy is approximately 3 mSv (equivalent to 30 chest X-rays). Please see attached radiation safety report for further information.

### 3.5. Impact of study results

A significant barrier to increased uptake of the LSAGB is the potential for increased bile reflux post-operatively, an area with a paucity of objective studies. Our study will provide additional data on the safety of the procedure, both generally and specifically relating to bile reflux, impacting surgical decision making in bariatric surgery. We hope to provide clinicians with clear evidence from which they can make informed decisions about the safety of the LSAGB.

With current data showing favourable results, including improved efficacy and decreased risk of complications, increased utilisation of the technique will result in improved short-term and long-term patient outcomes. Awareness of the incidence of bile reflux post-operatively will allow clinicians to treat any reflux early, preventing or at least delaying potentially detrimental health outcomes. These interventions will translate into decreased health expenditure, providing net health and economic benefit.

# 4. Study Plan and Design

**4.1. STUDY TYPE**

Prospective Cohort Study

**4.2. INCLUSION CRITERIA:**

Patients undergoing obesity surgery at the Royal Adelaide Hospital, The Queen Elizabeth Hospital, Burnside War Memorial Hospital, Western Hospital, Calvary Wakefield Hospital, Flinders Private Hospital or Ashford Hospital who have:

- Obesity of >5 years duration
- BMI>40 or >35 plus comorbidities
- Documented attempts at previous weight loss
- Age 18-60
- Good commitment to achieving weight loss
- Suitability for bariatric surgery

**4.3. EXCLUSION CRITERIA:**

Patients who have:

- Previously undergone obesity surgery
- Previously undergone gastric surgery
- Previously undergone gallbladder surgery
- Large abdominal hernias
- Pregnancy or are breast feeding
- Psychiatric illness
- BMI>65

**4.4. SAMPLE SIZE:**

*A sample size of 72 (24 in each of the 3 study arms) is required to find a significant difference in percentages of patients with bile reflux following 3 different types of operations.*

This was determined using a *Fisher's Exact Conditional Test for Two Proportions*, assuming a power of 80% and alpha=0.05. A difference of 45% between worst and best operation in terms of reflux was considered (5% vs 50%, which represents a 'best estimate' of bile reflux post-operatively due to lacking published data). Because there is clustering on surgeon in this data, an inflation factor (IF) was used to account for correlation within surgeon. Inflation

factor=1 + (m-1)p where m=cluster size and p=intra-class correlation coefficient (ICC) (https://www.newton.ac.uk/files/seminar/20110815152516051-152821.pdf). So IF for 5 surgeons and ICC=0.05 is 1.2. An additional 10% was then added to account for loss to follow-up. N per group was then multiplied by 3 (3 operation groups) to give total sample size needed.

A further 24 participants, from 1 surgical arm of the main trial, who have previously undergone surgical gallbladder removal (an exclusion criterion for the main trial) will also be recruited. This subset will allow us to compare the impact of gallbladder removal on the rate and severity of bile reflux for 1 operation. This sub-group of patients will not be subjected to the entire study protocol outlined below. They will only undergo the HIDA scan and the symptom questionnaire, but not the endoscopy.

## 4.5. METHODOLOGY:

### 4.5.1. RECRUITMENT

- Patients from either the outpatient department of CALHN public hospitals (Royal Adelaide and Queen Elizabeth Hospitals), or in the private consulting rooms of Mr Jon Shenfine, Mr. Justin Bessell, Mr Philip Game or Mr Jacob Chisholm (specialist weight loss surgeons), to be worked up for primary weight loss surgery will be invited to take part in the study
- If the patient meets the inclusion/exclusion criteria, the study will be discussed with the patient by their treating surgeon or member of the investigative team, and they will be provided with a pack containing:
    - A 'Participant Information Sheet' (Appendix 1)
    - A 'Participant Consent Form' (Appendix 2)
    - A 'Participant Details Form' (Appendix 3)
    - A 'GerdQ - Gastro-oesophageal reflux symptom questionnaire' (Appendix 4)
- If the potential participant would like further time to consider involvement, a follow-up meeting with a member of the investigative team will be arranged
- No financial inducement will be offered, however support for parking cost reimbursement will be offered

### 4.5.2. PRE-SURGICAL WORK UP

- Participants will undergo the following investigations pre-operatively:
    - Endoscopy, including:
        - Gastric (+/- oesophageal) biopsies
        - Gastric aspirates for biochemical analysis
    - Blood testing

        - Complete blood examination
        - Serum levels of glucose and HbA1c
        - Quantification of fasting lipids
    - Weight and height will be measured for body mass index
        - Hip and waist circumference will also be measured to evaluate central obesity index

#### 4.5.3. SURGERY

- Determination of the type of operation (LSAGB vs LSG vs LRYGB) to be performed will be at the discretion of the treating surgeon and informed patient preference.
- The operations will be performed by consultant oesophagogastric surgeons at the Royal Adelaide Hospital, The Queen Elizabeth Hospital, Western Hospital, Burnside War Memorial Hospital, Calvary Wakefield Hospital, Flinders Private Hospital or Ashford Hospital.

#### 4.5.4. POSTOPERATIVE CARE

- As per standard in-hospital pathways for clinical care following bariatric surgery

#### 4.5.5. FOLLOW-UP

- Participants will be contacted 4-6 weeks post-operatively to assess recovery and confirm continued participation in the study
- Participants invited at 6 months post-operatively to complete the same GerdQ Questionnaire (Appendix 4), in addition to a 'Investigations Tolerability Questionnaire' (Appendix 5)
- Participants who **have not had previous gallbladder surgery** will be invited to undergo the following investigations at 6 months post-operatively:
    - HIDA scan
        - To be performed at the Royal Adelaide Hospital in the Nuclear Medicine Department
        - Biliary scanning will be performed in all subjects following a 6-hour period of fasting and 24-hour abstinence from opiate containing medicine
        - Patients will be injected intravenously with 180 MBq $^{99m}$Tc DIDA and then dynamic scans will be performed for 100 minutes on a GE, discovery D670 gamma camera using a LFOV with the patient lying supine on the scanning bed
        - A lipid-rich 'fatty meal' (60mL of *Calogen*) will be ingested orally after 30 minutes to stimulate gallbladder emptying

        - Following this a SPECT CT scan will be performed of the abdomen for more accurate anatomical localization of activity
        - Delayed imaging may be performed depending on biliary function but only a single low-dose CT will be performed.
    - Endoscopy
        - Gastric (+/- oesophageal) biopsies
        - Gastric aspirates for biochemical analysis
    - Blood testing
        - Complete blood examination
        - Serum levels of glucose and HbA1c
        - Quantification of fasting lipids
    - Weight and height will be measured for body mass index
        - Hip and waist circumference will also be measured to evaluate central obesity index
- Participants who **have undergone previous gallbladder surgery** will only undergo the HIDA scan and symptom questionnaire.
- At 6 months after surgery, if a patient experiences clinically troublesome symptoms and a deterioration in symptom score is noted, then the patient will be offered oesophageal manometry and pH-impedance testing to evaluate swallowing function and to assess the presence of reflux in the oesophagus.

# 5. Outcomes

**5.1. PRIMARY OUTCOMES:**

- Scintigraphic evaluation of presence and degree of gastro-oesophageal bile reflux
- Impedance-based characterisation of oesophageal acidity, composition, duration and extent of oesophageal reflux
- Determination of presence of bile acids in gastric pouch and oesophageal fluid aspirates
- Determination of bile-acid induced gastric and oesophageal histological tissue changes

**5.2. SECONDARY OUTCOMES:**

- Biliary gastritis/oesophagitis/other markers of mucosal inflammation in the stomach and oesophagus
- Weight Loss/BMI
- Metabolic changes
- Quality of life
- Operative time
- Rate of conversion from laparoscopic to open operative technique
- Post-operative surgical complications such as, anastomotic leak, infection, cardio-respiratory events, kidney injury, venous thromboembolism, and so on.
- Length of hospital stay
- Patient tolerability of investigations (hepatobiliary scintigraphy, oesophageal reflux tests and endoscopy with aspiration and biopsy), with regard to personal comfort prior to, during, and after the investigation.

# 6. Resource and Funding Considerations

### 6.1. RESOURCE CONSIDERATIONS

This project will be conducted using facilities in the Departments of Surgery at the Royal Adelaide, Queen Elizabeth and Calvary Wakefield Hospitals and Department of Nuclear Medicine at the Royal Adelaide Hospital and will be supported by existing departmental research funds.

### 6.2. FUNDING CONSIDERATIONS

- Costs of manometry and pH-impedance testing
    - These routine diagnostic tests, where performed post-operatively, will be performed and billed in accordance with clinical practice for ambulatory outpatient procedures
- Costs of hepatobiliary scintigraphy
    - These costs will be absorbed by the Royal Adelaide Hospital Department of Nuclear Medicine
- Cost of follow-up endoscopy
    - Follow up endoscopy will be performed to ensure that patients have not developed a hiatal hernia as a result of operative manipulation. In all bariatric surgery there is manipulation of the proximal stomach and a hiatal hernia may occur, increasing the risk of post-operative reflux.

The study investigators have no financial interest in the outcome of the research project and are not receiving any support from a commercial company or other financial support. There are no conflicts of interest to declare.

# 7. Ethical Considerations

### 7.1. STUDY DESIGN

Although a prospective, randomized controlled trial would provide scientific rigour, we have elected to undertake a cohort study. Individual patient factors significantly impact the type of weight loss operation offered by surgeons, due to potential long-term morbidity, and thus clinical equipoise is lacking for randomization. Involvement in the study will not impact on the type of operation offered and investigator bias will be minimised through informed patient consent after in-depth discussion about available treatment options.

### 7.2. PRE-OPERATIVE WORKUP

The current standard pre-operative work-up for patients at the Royal Adelaide and Queen Elizabeth Hospitals is for biometric measurements (height, weight and BMI), endoscopy, routine blood testing and selective use of oesophageal manometry and 24-hour pH-impedance reflux testing. All cohorts in the study will undergo these standard investigations, with selective use of manometry and pH-impedance testing as described earlier.

### 7.3. POST-OPERATIVE FOLLOW-UP

Post-operative follow-up investigations will be performed at 6-months post-operatively. This time frame will provide sufficient time for patients to recover from their operation and returned to their usual activities.

Standard follow-up investigations after bariatric surgery at the Royal Adelaide and Queen Elizabeth Hospitals are biometric measurements (height, weight and BMI), routine blood testing and assessment of symptoms.

In addition to the above standard follow-up, all participants of this study will undergo hepatobiliary scintigraphy to evaluate bile reflux. This investigation has been used previously to assess for reflux (see section 3 of this protocol) and has more recently been used to evaluate reflux after weight loss surgery (24). For the specific safety considerations regarding radiation exposure with hepatobiliary scintigraphy, see section 8 of this protocol.

### 7.4. RISKS / ADVERSE EFFECTS OF INVESTIGATIONS

#### 7.4.1. HIDA SCINTIGRAPHY

HIDA scintigraphy is a routine diagnostic test in the Royal Adelaide Hospital and Queen Elizabeth Hospital and performed in the Department of Nuclear Medicine numerous times per week. HIDA is often used to assess gall-bladder emptying, however it has also been used previously to assess for bile reflux. The research protocol will use standard clinical practices for assessment of bile reflux.

The risk of radiation exposure is discussed in section 8 of this protocol. Exposure to the intravenous tracer may induce an allergic response, at which time the investigation will be aborted and immediate medical assistance sought. The prolonged supine positioning (100 minutes) may cause some back discomfort for patients. Those with pre-existing back pain will be counseled prior to the study and prescribed analgesia as appropriate.

### 7.4.2. ENDOSCOPY

Endoscopy is a routine diagnostic test in the Royal Adelaide Hospital and Queen Elizabeth Hospital and performed in the Gastrointestinal Investigation Unit daily. The research protocol will use the standard clinical practices for this procedure.

Some minor discomfort or gagging may occur during the procedure, which is minimised by the use of intravenous opioid analgesia and sedation (benzodiazepine) as per standard clinical practices. Some minor bleeding can occur after collection of gastric and oesophageal biopsies. Patients on blood thinners will be instructed not to take them for a period prior to the endoscopy, after consultation with the prescribing clinician.

### 7.4.3. OESOPHAGEAL MAMOMETRY AND IMPEDANCE-PH TESTING

Oesophageal manometry and impedance-pH testing are routine diagnostic tests in the Royal Adelaide Hospital and Queen Elizabeth Hospitals and performed in the Oesophageal Function Laboratory daily. The research protocol will use the standard clinical practices for this procedure.

As with routine diagnostic manometry and impedance testing, after a 6hr fast, topical nasal anaesthesia (5% co-phenylcaine) will be applied before passing trans-nasally the oesophageal motility catheter lubricated with water-based jelly (K-Y jelly). Some minor throat discomfort or gagging may occur as the catheter passes the pharynx, but once positioned, adaptation occurs and there

is minimal or no discomfort. Similarly, minor transient discomfort may be experienced with the withdrawal (removal) of the catheter.

# 8. Specific Safety Considerations

**8.1.** RADIATION EXPOSURE

The subjects will be exposed to a diagnostic dose of radiation relating to the performance of the $^{99m}$Tc DIDA scan and also a low-dose CT scan of the abdomen as part of the SPECT/CT study. A radiation safety report is attached and the calculations indicate that subjects will be exposed to a dose of 9.2mSv per study. Please see report for full details.

# 9. Analysis and Reporting of Results

**Sample analysis**

- Gastric biopsies will be reviewed by a qualified histopathologist within SA Pathology to assess:
    - Markers of tissue inflammation
    - Markers of DNA damage
    - Presence of bile acids
- Gastric aspirates will be analysed in the appropriate means by SA Pathology to assess:
    - Presence of bile acids
    - Concentration of bile acids
    - Identification of individual bile acids

**$^{99m}$Tc DIDA scan analysis of DGER**

- The rate of Hepatobiliary excretion of tracer and amount of DGER will be calculated from the scans using dynamic imaging with region of interest calculations over the liver, duodenum, gastric remnant and oesophagus.

**Statistical Analysis of data**

- Statistical analysis will be undertaken for the primary outcomes relating to the three types of bariatric surgery being utilised in current clinical practice using commercially available statistical programs in consultation with professional statistical advice from our Unit's established connection with a health research statistician.
    - Normally distributed data will be reported as mean ± standard error of the mean and statistical comparisons between groups will be made using parametric statistical tests.
    - Data that is not normally distributed, will be analysed using non-parametric statistics with reporting of median (interquartile range) and comparisons by applying non-parametric statistical tests with post-hoc analysis for differences across the 3 surgical groups.
    - Proportionate data will be compared using tests such as a Fisher's exact test or $X^2$ test.
- The results will be presented at a relevant scientific forum and subsequently be submitted for publication in a peer-reviewed medical journal.

# 10. Data and Biological Sample Storage

### 10.1. PARTICIPANT INFORMATION

- Personal data (name, contact details, etc.) will be recorded on the *Participant Details Form* (Appendix 3).
- Participants will be allocated individual numerical identifiers that will be used during the study for de-identification.
- The *Participant Details Forms* (Appendix 3) will be secured in a locked filing cabinet or similar, within the Department of Surgery at The Queen Elizabeth Hospital.

### 10.2. QUESTIONNAIRES

- Data from 'Reflux Symptom Score' and 'Investigation Tolerability' questionnaires will be stored electronically and kept securely on a password-protected computer in the Royal Adelaide Hospital. These data will also be backed up to a suitable secure CALHN device or server.
- Physical questionnaires will be secured in a locked filing cabinet or similar, within the Department of Surgery at The Queen Elizabeth Hospital.

### 10.3. BIOLOGICAL SAMPLES (BLOOD, TISSUE, BODILY FLUIDS)

- Samples will be stored in accordance with standard practice by and within SA Pathology.
- Data from laboratory analysis of blood, tissue and body fluid samples will be stored electronically and kept securely on a password-protected computer in the Royal Adelaide Hospital. These data will also be backed up to a suitable secure CALHN device or server.

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# 12. Proposed Commencement / Duration

**Commencement Date:** February 2018

**Duration:**

- Patient recruitment: February 2018 – July 2020
- Patient follow-up: Six months after last operation is performed (around August 2020)

# 13. Signatures of Investigators

The protocol has been read and endorsed by all of the investigators, identified as:

| | | |
|---|---|---|
| Dr. Thomas Eldredge | _____ | ___/___/____ |
| University of Adelaide | Signature | Date |
| Dr. Jennifer Myers | _____ | ___/___/____ |
| CALHN | Signature | Date |
| A/Prof. George Kiroff | _____ | ___/___/____ |
| CALHN | Signature | Date |
| Mr. Jon Shenfine | _____ | ___/___/____ |
| CALHN | Signature | Date |
| A/Prof Dylan Bartholomeusz | _____ | ___/___/____ |
| CALHN | Signature | Date |
| Ms. Madison Bills | _____ | ___/___/____ |
| CALHN | Signature | Date |
| Dr. Mark Harris | _____ | ___/___/____ |
| CALHN | Signature | Date |
| Dr. Matt Watson | _____ | ___/___/____ |
| CALHN | Signature | Date |

# 14. Appendices

Appendix 1: **Participant Information Sheet**

Appendix 2: **Participant Consent Form**

Appendix 3**: Participant Details Form**

Appendix 4: **GerdQ - Gastro-oesophageal reflux symptom questionnaire**

Appendix 5: **Investigation Tolerability Questionnaire**