The effect of increased sodium intake with a carbohydrate-rich meal on glucose homeostasis in subjects without diabetes after bariatric surgery (SALT): A proof-of-concept, randomised, open-label, crossover study.

Short title: The SALT Study

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AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Details of change made
Substantial Amendment 1	protocol version 3.0 dated 15.10.2020	Protocol amended to include Macronutrients of pre-packed meal participants will receive for the Mixed Meal Tolerance Test. Removal of product branding of orange juice listed within previous protocol.
NSA 1	protocol version 3.1 dated 21.04.2021	Timelines mentioned within protocol amended due to impact of COVID-19 and study being on pause.
NSA 2	protocol version 3.2 dated 05.07.2021	Amendment to include Professor David Bowrey as a Co-investigator and amending the Patient Invitation Letter. Further minor changes made to the protocol around consistency of visit windows to align with page reference numbers as stated within the protocol. Including advertising through the use of Research registries, databases and charities such as Obesity UK to further promote the study and support recruitment.
NSA 3	protocol version 3.3 dated 07.12.2021	Amendment to modify current exclusion criteria statement around use of oral steroids, intolerance to mixed meal tolerance test . Minor changes to consent form to include screening ID and updated study thank you letter with new study end date.
NSA 4	protocol version 3.4 dated 10.02.2022	Amendment to modify current exclusion criteria statement to support recruitment. Study team have also updated the recruitment strategy to extend CRN support outside of Leicestershire. Recruitment strategy to include recruitment of those who have been discharged from private hospitals and Hospitals outside of Leicestershire to be set up as PIC sites. Updated study MMTT wording for % macronutrients. Within this amendment study team have also updated end of study timelines. Amendment to also update supporting study documents such as PIS, PIC invitation letter and patient thank you letter to reflect these changes. Removal of TMG meeting and amended to OP's Meetings.
NSA 5	Protocol v3.5 13.06.2022	Amendment to update current study recruitment end date and overall study end date within protocol and patient letters. Update to recruitment strategy within protocol to extend use of PIC sites (primary and secondary care) into the West Midlands. Update to appointment letters to reflect updated travel re-imbursement costs.
NSA 6	Protocol v3.6 09.09.2022	Within this amendment, we have revised the study exclusion criteria to clarify that any participants taking part in a CTIMP within

		<1 month of screening will be excluded. We have extended the study recruitment end date to April 2023 and overall study end date to 31st May 2023 We have also included University Hospitals of Birmingham and Luton & Dunstable University Hospital (Bedfordshire Hospitals NHS Trust) as PIC sites. The patient thank you letter has been updated to reflect the new study end date.
NSA 7	Protocol v3.7 19.05.2023	Within this amendment we have extended the recruitment end date to 14.09.2023 and the overall study end date to 30.09.2023. The patient thank you letter has been updated to reflect the new overall study end date.
NSA 8	Protocol v3.8 17.11.2023	Within this amendment we are extending the study end date to 30.04.2024 to allow time for statistical analysis.

1. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
AUC	Area Under the Curve
BP	Blood Pressure
CRF	Case Report Form
CTIMP	Clinical Trials of Investigational Medicinal Products
EC	Ethics Committee (see REC)
GCP	Good Clinical Practice
GP	General Practitioner
LDC	Leicester Diabetes Centre
MMTT	Mixed Meal Tolerance Test
NHS	National Health Service
NaCl	Sodium chloride (Salt)
PIL	Participant/ Patient Information Leaflet
PHH	Postprandial Hyperinsulinaemic Hypoglycaemia
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RYGB	Roux-en-Y Gastric Bypass
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
UHL	University Hospitals of Leicester NHS Trust

2. SUMMARY – PROTOCOL SYNOPSIS

Background: Postprandial hyperinsulinaemic hypoglycaemia (PHH) is a common and disabling complication after Roux-en-Y gastric bypass (RYGB), one of the most commonly performed bariatric procedures worldwide. The treatment options for PHH are limited. The underlying pathophysiology of PHH after RYGB is unclear, however the altered intestinal glucose absorption and the rapid nutrient delivery at the distal small intestine postoperatively have a major impact on the glucose homeostasis after RYGB and are important contributing factors to PHH.

Recent studies in animals (mini-pigs) demonstrated that the absorption of dietary glucose after RYGB takes place mainly at the common limb and is reduced in the alimentary limb due to a functional defect of SGLT-1 at the sodium deprived postoperative alimentary limb. The addition of 2g sodium chloride (NaCl) at the meal of mini-pigs increased glucose absorption at the alimentary limb and increased the postprandial glucose levels [including nadir (lowest) glucose levels] after RYGB, suggesting that SGLT-1 functional defect in alimentary limb can be restored with the addition of sodium. If this is the case also in humans, increased sodium intake with a carbohydrate-rich meal could be a treatment option for PHH after BS.

Objective of the study: To investigate the effect of increased sodium intake with a carbohydrate-rich meal on glucose homeostasis in patients without diabetes after RYGB.

Aim of the study: The aim of study is to investigate the effect of increased sodium intake with a carbohydrate-rich meal on glucose levels, insulin and gut hormones in patients without diabetes after Roux-en-Y gastric bypass surgery.

Hypothesis: We hypothesize that increased sodium intake with a carbohydrate-rich meal in patients without diabetes after RYGB will increase the postprandial and nadir glucose levels after RYGB through a more physiological and gradual absorption of the dietary glucose at the proximal small intestine.

Design of the study: The study will be a proof of concept, open-label, crossover study. Eligible subjects without diabetes after RYGB (n=14) will be randomised to one of the following treatment sequences: a) standardised mixed meal tolerance test (MMTT) with 2g of table Salt (NaCl) or b) standardised MMTT without additional 2g of table Salt (NaCl). On the morning of day 0 of the study (visit 1), after an overnight fast, a cannula will be inserted and participants will have a 3-hour MMTT with or without additional 2g of table Salt (NaCl), based on treatment sequence. Standardised MMTT will consist of 170mls of smooth orange juice (where the 2g of table Salt will be diluted for those on the "additional table Salt" treatment sequence) followed by a pre-packed meal. The mixed meal (orange juice plus the pre-packed meal) which approximately comes to 300kcal , broken down into an approximate % macronutrient composition of 61% carbohydrates, 26% fat and 9% protein and 0.15g of Salt.

Blood samples for glucose, insulin, c-peptide and GLP-1 will be collected at fasting state (immediately before MMTT meal) and 15', 30', 60', 90', 120', 150', 180' after meal ingestion. Moreover, validated questionnaires regarding dumping symptoms and hypoglycaemia symptoms will be completed at the same time points with blood collection.

After the 7-day washout period, participants will undergo a second standardized MMTT (Visit 2, day 7 of the study) with or without added 2g of table Salt (NaCl) (opposite to first treatment option) and procedures will be repeated as described in Visit 1.

Primary outcome: The difference in nadir (lowest) glucose levels after the standardised MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB.

Secondary outcomes:

Difference in Area Under the Curve $(AUC)_{(0\text{-}180)}$, fasting and peak glucose levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB.

Difference in $AUC_{(0\text{-}180)}$ insulin, fasting and peak insulin levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in $AUC_{(0\text{-}180)}$ GLP-1, fasting and peak GLP-1 levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in AUC $_{(0\text{-}180)}$ c-peptide, fasting and peak c-peptide levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio AUC $_{(0\text{-}180)}$ insulin/AUC$_{(0\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio AUC $_{(0\text{-}30)}$ insulin/AUC $_{(0\text{-}30)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio AUC $_{(60\text{-}180)}$ insulin/AUC $_{(60\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio AUC $_{(0\text{-}180)}$ c-peptide/AUC $_{(0\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio AUC $_{(0\text{-}30)}$ c-peptide/AUC $_{(0\text{-}30)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio AUC $_{(60\text{-}180)}$ c-peptide/AUC $_{(60\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio of maximum/minimum plasma glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in AUC $_{(0\text{-}180)}$ of Sigstad score and peak Sigstad score after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference between AUC $_{(0\text{-}180)}$ of Edinburgh Hypoglycaemia Scale score and peak Edinburgh Hypoglycaemia Scale score after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

The Amount of glucose (in grams) needed to restore euglycaemia between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

The number of MMTTs needed to be terminated early because of hypoglycaemia (blood glucose or capillary glucose levels ≤3.0mmol/l) with and without additional NaCl 2g after RYGB

3. BACKGROUND AND RATIONALE

Obesity is a major national and global public health challenge which is associated with significant co-morbidities and increased mortality (1). In UK, more than 25% of the population is obese and approximately 10% suffers from severe and complex obesity (defined as BMI≥35 kg/m^2 with obesity related comorbidities) (2, 3). Bariatric surgery is the most effective method to achieve significant long-term weight loss and weight maintenance in patients with severe and complex obesity (4).

Roux-en-Y gastric bypass (RYGB) is one of the most commonly performed bariatric surgeries in UK and account for approximately 40% of bariatric procedures worldwide (5). Despite successful weight loss and weight maintenance, some long-term complications can develop after RYGB, such as nutritional and vitamin deficiencies, early dumping syndrome and postprandial hyperinsulinaemic hypoglycaemia (PHH) (6-8).

Postprandial hyperinsulinaemic hypoglycaemia (PHH) is a condition characterized by hypoglycaemic symptoms occurring 1-3 hours after a meal accompanied by a low venous glucose value, typically preceded by a high rise in both glucose and insulin concentration (6-8). PHH has been described since 1940s as complication of gastric resection in patients suffering from peptic ulcers and was named “late dumping”. The condition has recently warranted further attention due to the increased number of bariatric procedures worldwide. It is of note that recurrent PHH after RYGB is associated with reduced quality of life, high degree of functional disability (inability to work, drive, care for others) and weight regain (9,10). In addition, an increased rate of accidental deaths, syncopal episodes and seizures among patients who have undergone RYGB has been reported, and it is speculated that this could be due to neuroglycopenic symptoms as result of severe PHH (11,12).

The incidence of hypoglycaemia during daily life ranges between 17-75% after RYGB, depending on the definition of hypoglycaemia, the population studied, and the diagnostic tool used for assessment of hypoglycaemia (2,13-15). Risk factors for developing symptoms of postprandial hypoglycaemia include young age, female gender, no history of diabetes preoperatively, longer time since surgery and significant weight loss postoperatively (14,15,16).

Treatment options for PHH after RYGB are limited and patients are most commonly encouraged to follow dietary modifications consisting of small, frequent and low in carbohydrate meals. Although potentially efficient, a low carbohydrate diet presents large compliance problems and may not be applicable to all patients suffering from PHH (8). Medical treatments include mainly acarbose and somatostatin analogues (17-22), but their limited effectiveness, side effects and cost limit their use (8). In cases of severe and refractory PHH after RYGB despite diet modifications and medical treatments, treatment options include

complex surgical procedures such as partial or total pancreatectomy, reversal of RYGB and gastric outlet restriction. All of these surgical treatment options have limited evidence, inconclusive results, increased risk of complications and some of these options are associated with the disadvantageous risk of weight regain. Thus, alternative treatments are needed; however the development of effective treatments for PHH after BS requires detailed understanding of the underlying mechanisms of the condition.

Currently, the underlying pathophysiology of PHH after BS is incompletely understood, but the most likely explanation is that it is a physiological result of altered glucose absorption at the small bowel postoperatively (23,24). The normal gradual absorption of carbohydrate does not occur when a high in carbohydrates meal is ingested after RYGB (24-26) due to the rapid gastric emptying (27) and the bypass of the ingested nutrients away from the stomach and duodenum (25,26). The rapid arrival and absorption of a disproportionately greater amount of carbohydrates at the distal small bowel leads to a prominent early spike in postprandial glucose levels and subsequently triggers a corresponding over-secretion of insulin and gut hormones contributing to glucose homeostasis such as Glucagon Like Peptide-1 (GLP-1) levels leading to PHH (23,24,28,29). Indeed, people who experience PHH after RYGB have increased endogenous insulin secretion and increased GLP-1 secretion compared to those without PHH (28,29). Also, in accordance with this theory, feeding through gastrostomy tube to the remnant stomach after RYGB can lead to remission/improvement of PHH, reduce peak glucose levels, and reduce GLP-1 and insulin secretion (23).

Sodium glucose co-transporters-1 (SGLT-1) are responsible for dietary glucose absorption at the intestine and are highly expressed in the proximal part of the small intestine (30-32). There is an increasing body of evidence that SGLT-1 is also a sensor linking glucose to acute GLP-1 release (32,33). Recent studies in mini-pigs demonstrated that the absorption of dietary glucose after RYGB takes place mainly at the common limb and is reduced in the alimentary limb (34), probably due to a functional defect of SGLT-1 at the sodium deprived postoperative alimentary limb (34,35). On the other hand, SGLT-1 remains functional at the common limb where sodium-rich bile is added (34). In contrast to these findings, in vitro studies have demonstrated that SGLT-1 expression and glucose transport activity is increased at the alimentary limb after RYGB in rats (36), however, it should be noted that this experiment has been performed in a sodium-rich solution (Ringer bicarbonate solution) which could explain why glucose uptake capacity of the alimentary limb was restored in that case (34).

Indeed, the addition of 2g sodium chloride (NaCl) at the meal of mini-pigs increased also the glucose absorption at the alimentary limb and increased the postprandial glucose levels [including nadir (lowest)

glucose levels] after RYGB (34), suggesting that SGLT-1 functional defect in alimentary limb can be restored with the addition of sodium. So, increased sodium intake with a carbohydrate rich meal could be a potential treatment for PHH. Indeed, previous studies in healthy humans without previous bariatric surgery have demonstrated that increased NaCl intake with a carbohydrate rich meal can increase postprandial glucose levels (37) due to increased intestinal absorption; however, currently there is no data on the effect of increased NaCl intake with a meal on glucose homeostasis after RYGB.

This study will be the first one to investigate the effect of increased sodium intake with a standardised carbohydrate rich meal on glucose homeostasis in subjects without diabetes after bariatric surgery.

Research Hypothesis: The research hypothesis is that increased sodium intake with a carbohydrate rich meal will increase the postprandial and nadir glucose levels after RYGB through more physiological and gradual absorption of the dietary glucose at the proximal small intestine.

4. OBJECTIVES

The aim of the study is to investigate the effect of increased sodium intake with a carbohydrate-rich meal on glucose levels, insulin and gut hormones in patients without diabetes after Roux-en-Y gastric bypass surgery.

The objectives of the current proof of concept, randomised, open label, crossover study is to investigate the effect of increased NaCl intake with a rich in carbohydrate meal after RYGB on

1) fasting, peak, nadir and postprandial glucose levels
2) fasting, peak and postprandial insulin levels
3) fasting, peak and postprandial c-peptide levels
4) fasting, peak and postprandial GLP-1 secretion during a standardised mixed meal tolerance test (MMTT)
5) symptoms suggestive of postprandial hypoglycaemia
6) symptoms suggestive of dumping syndrome

5. STUDY DESIGN

5.1 Trial Summary

This study is a proof-of-concept, randomised, open label, crossover study conducted in male and female participants without diabetes who have undergone Roux-en-Y gastric bypass (RYGB).

Participants will be randomised to one of the following two treatment sequences at baseline:-

- Group 1: will receive a standardised MMTT with 2g of table salt at visit 1, and then a standardised meal without additional table salt at visit 2.
- Group 2: will receive a standardised MMTT without addition of 2g table salt at visit 1, then a set meal with 2g table salt at visit 2.

The participant flowchart is illustrated in Figure 1 (page 18).

Participants will attend a screening (familiarisation) visit prior to the start of the study followed by 2 visits over 8 days.

The first visit (visit 0) is the Screening (Familiarisation) Visit and will occur not more than 14 days nor less than 1 day before the Visit 1 (Baseline visit). Visit 0 (approximately 2 hours) will involve an assessment of inclusion/exclusion criteria, an explanation of study procedures and obtaining verbal and written consent from participants by a trained healthcare professional. In addition, blood will be taken for HbA1c, Full blood count (FBC), renal function and liver function as part of investigation for exclusion criteria. A urine pregnancy test will also take place for all female participants of child bearing potential. These samples will all be processed at the pathology laboratory within the Leicester General Hospital. In addition demographic information, past medical/surgical history, concomitant medication, medication history will also be collected at this visit. A general physical examination will be performed by a trained delegated clinician.

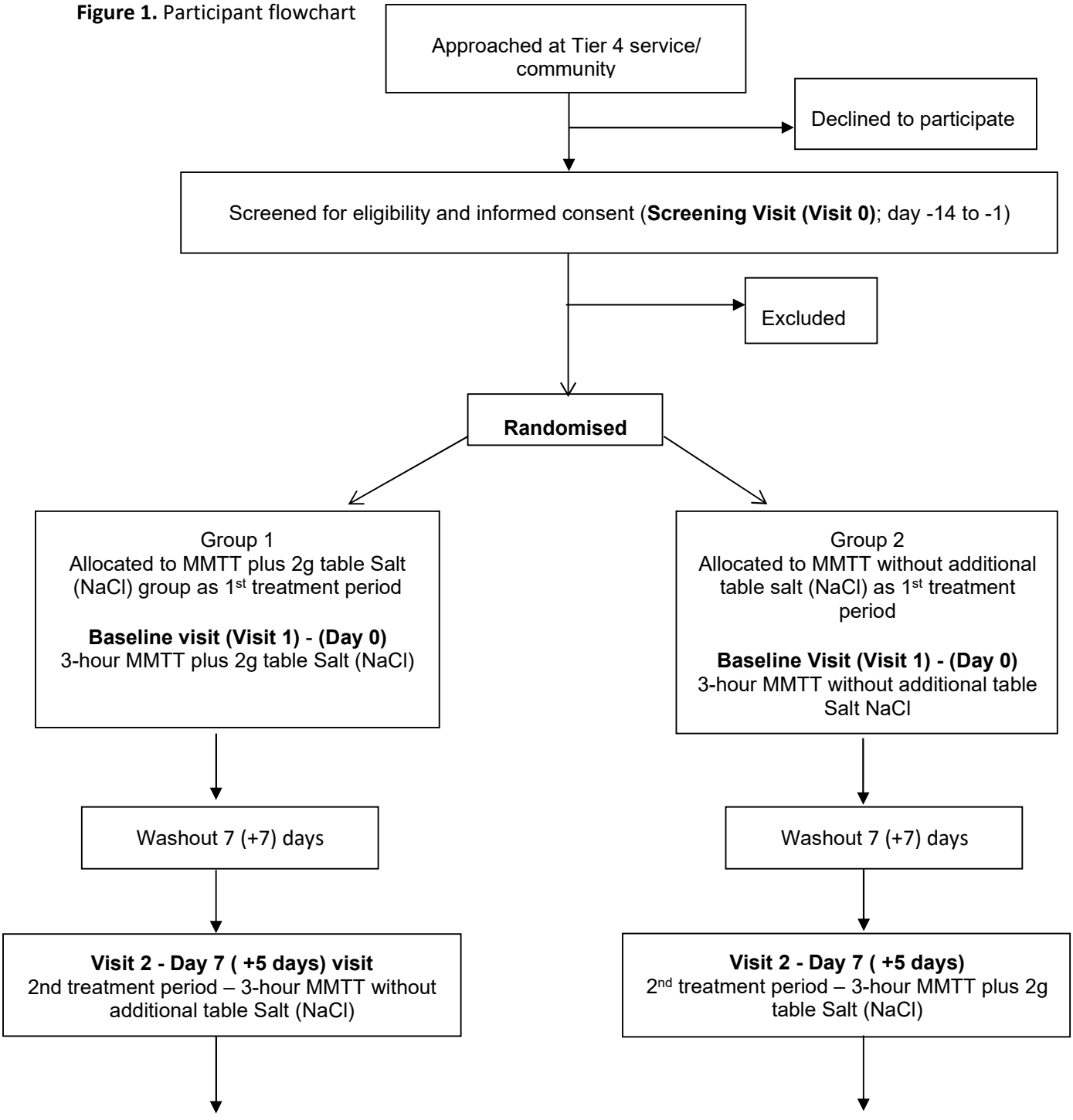
Visit 1 is the Baseline visit lasting approximately-5 hours and will take place at Leicester Diabetes Centre. Randomisation to one of the two treatment sequences will take place during this visit. Anthropometrics (Weight (including body fat %), Height, blood pressure (BP), Pulse rate) will be measured. Changes in medications since screening will be documented. Urine pregnancy test will be performed in all women of childbearing potential. A cannula will be inserted to allow multiple blood samples collection. Participants randomised to standardised MMTT plus 2g additional table Salt (NaCl) treatment sequence will be asked to

drink first 170mls of orange juice with 2g of table Salt (NaCl) diluted into this and then consume the standardised meal) under supervision. Participants randomised to standardised MMTT without additional Salt (NaCl) will be asked to drink 170mls of orange juice (without added table Salt) and then consume the standardised meal under supervision. Blood samples will be collected in the fasting state (immediately before MMTT) and at 15', 30', 60', 90', 120', 150' and 180' after MMTT ingestion for measurement of glucose, insulin, c-peptide and GLP-1. Questionnaires on dumping symptoms as well as questionnaires on hypoglycaemia symptoms will be completed during the MMTT at the same time points as blood collection with the aid of the research team. For the 24 hours before study visits 1 and 2, participants will be asked to refrain from: completing any **moderate to vigorous** form of physical activity and consuming any alcohol.

Visit 2 (occurs 7 days after visit 1) lasts approximately 5 hours and is similar to visit 1. The only change is that participants who were initially allocated to standardised MMTT plus 2g additional table Salt (NaCl) will now receive the standardised MMTT without additional table salt (NaCl) sequence. Conversely, those who were initially allocated to receive the standardised MMTT without additional table salt, (NaCl) will receive the standardised MMTT with the table salt (NaCl) added. The procedures will be repeated as per Visit 1 for both groups. Urine pregnancy test will be performed to all women of childbearing potential at Visit 2.

Safety Call 1 (occurs 7 days after Visit 2) is a telephone call to the patient. Participants will be asked to report any adverse events between Visit 2 and the day of the Safety Call 1. Safety call 1 is the end of the study.

Figure 1. Participant flowchart



Safety call (Telephone) – Day 14 (± 5 days) Ask participant regarding side effects End of the study	**Safety call (Telephone) – Day 14 (± 5 days)** Ask participant regarding side effects End of the study

MMTT: Mixed meal tolerance test, NaCl: Sodium chloride

5.2 Setting and timeframe

The study will be co-ordinated within the University Hospitals of Leicester NHS Trust and University of Leicester and clinical measurement sessions will be co-ordinated by the appointed research team based at the Leicester Diabetes Centre, University Hospitals of Leicester NHS Trust, in collaboration with academic partner Loughborough University who are part of the NIHR Leicester Biomedical Research Centre (BRC). There are state-of-the-art laboratory facilities for analysing glucagon like peptide-1 (GLP-1) levels at the National Centre for Sports and Exercise Medicine at Loughborough University run by Prof David Stensel. Measurement of insulin and c-peptide will take place by experienced research scientists at Leicester Diabetes Centre (LDC). Plasma glucose will be measured at the pathology lab of the University Hospitals of Leicester NHS Trust at Leicester General Hospital.

Recruitment will take place at Tier 4 clinics (post-bariatric surgery clinic) at University Hospitals of Leicester NHS Trust as well as from the primary care (GP practices), secondary care (secondary care databases, Bariatric surgery follow up clinics, etc). Recruitment will also take place within the community and community events. Once the study has commenced, recruitment will be open until 14th September 2023, with the study expected to close end of September 2023. The statistical analysis of the study will be completed by 30.04.2024.

5.3 Primary and Secondary Endpoints

Primary endpoint

The difference in nadir (lowest) glucose levels after the standardised MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB.

Secondary Endpoints

The secondary endpoints below will be measured at the time points defined in Table 1 (pages 32-33).

Difference in Area Under the Curve $(AUC)_{(0\text{-}180)}$, fasting and peak glucose levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB.

Difference in $AUC_{(0\text{-}180)}$ insulin, fasting and peak insulin levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in $AUC_{(0\text{-}180)}$ GLP-1, fasting and peak GLP-1 levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in $AUC_{(0\text{-}180)}$ c-peptide, fasting and peak c-peptide levels after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio $AUC_{(0\text{-}180)}$ insulin/$AUC_{(0\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio $AUC_{(0\text{-}30)}$ insulin/$AUC_{(0\text{-}30)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio $AUC_{(60\text{-}180)}$ insulin/$AUC_{(60\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio $AUC_{(0\text{-}180)}$ c-peptide/$AUC_{(0\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio $AUC_{(0\text{-}30)}$ c-peptide/$AUC_{(0\text{-}30)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio $AUC_{(60\text{-}180)}$ c-peptide/$AUC_{(60\text{-}180)}$ glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in the ratio of maximum/minimum plasma glucose after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference in $AUC_{(0\text{-}180)}$ of Sigstad score and peak Sigstad score after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

Difference between $AUC_{(0\text{-}180)}$ of Edinburgh Hypoglycaemia Scale score and peak Edinburgh Hypoglycaemia Scale score after MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

The Amount of glucose (in grams) needed to restore euglycaemia between the two treatment options (additional NaCl 2g vs no additional NaCl) after RYGB

The number of MMTTs needed to be terminated early because of hypoglycaemia (blood glucose or capillary glucose levels ≤3.0mmol/l) with and without additional NaCl 2g after RYGB

6. TRIAL PARTICIPANTS

6.1 Overall Description of Trial Participants:

Male and female subjects who are ≥ 18 years old, without a diagnosis of diabetes and at least one year after RYGB (n=14) will be recruited. Recruitment strategies will include identifying participants who meet the following inclusion/exclusion criteria from within primary and secondary care settings and the community.

6.2 Inclusion Criteria

- Aged≥18 years old but less than 75 years old
- Subjects ≥1 year after gastric bypass (RYGB)
- Able to understand written and spoken English
- Able to give informed consent

6.3 Exclusion criteria:

- Use of any glucose-lowering medication (including insulin)
- Adrenal insufficiency and/or substitution with glucocorticoids
- eGFR≤45 ml/min/1.73m^2
- Recent active infection (an active infection will be any infection over the last 10 days)
- Current use or history of treatment within 6 weeks with systemic glucocorticoids (oral or injectable) not including use of topical (e.g. eye drops or topical creams) or inhaled glucocorticoids.
- Known primary or secondary hyperaldosteronism
- Severe hypertension during screening visit (Systolic Blood Pressure >180mmHg as average of 3 measurements)
- Established diagnosis of congestive heart failure
- Significant peripheral oedema on clinical examination at screening visit
- People with allergy (e.g. milk protein allergy) or severe intolerance to the mixed meal test as assessed by the clinician (e.g. severe lactose and gluten intolerance)
- People following a vegan diet (mixed meal tolerance test not suitable for those following a vegan diet)
- Other bariatric procedure except of RYGB (except of gastric band which has been removed
- Previous revisional bariatric surgery (except of gastric band which has been removed)
- Currently pregnant or breastfeeding
- Diagnosis of type 1 diabetes
- Current diagnosis of type 2 diabetes (defined as HbA1C ≥6.5% at screening blood tests or HbA1C <6.5% at screening bloods but on glucose lowering medications over last 3 months)

- Patients with diagnosis of Epilepsy
- HbA1C ≥6.5% or ≥48 mmol/l at screening blood tests
- Haemoglobin (Hb) <100 g/L at screening blood tests
- Participating in a Clinical Trial of Investigational Medicinal Product (CTIMP) within <1 month of screening.
- Having a formal previous diagnosis of postprandial hypoglycaemia
- Being on acarbose, diazoxide, octreotide or other treatment for postprandial hypoglycaemia
- Any concurrent condition, in the judgment of investigator and/or sponsor, that could interfere with the safety and study conduct or interpretation of study results

If the study clinician and/or Investigator deems it clinically inappropriate to include a potential participant in the study, that potential participant will deemed 'not eligible' and will not be enrolled/consented to the study (i.e., they will be classed as a screen failure and recorded as such on the visit CRF and in their medical notes). Clinicians will consider any emerging safety concerns throughout the duration of the study and subsequent eligibility of participants.

7. STUDY PROCEDURES

7.1 Screening and Eligibility Assessment/Recruitment

Information describing the study (PIL) will be sent to individuals meeting the eligibility criteria at least 24 hours before a planned visit. The direct clinical/healthcare team will have sole responsibility for accessing patient information stored on the hospital system and performing database searches for the sole purpose of identifying potential participants and inclusion and exclusion criteria. Consultants/Specialist Registrars, dieticians or research healthcare professionals may also speak directly to possible participants in clinic and/or give them a PIL to which the patient can reply directly to the study team. The study information pack will include a reply slip, where interested parties will have the option to discuss the study and ask questions directly to a member of the research team either after the routine hospital visit or during a separate meeting. Those agreeing to participate will be asked to provide written consent to the study.

One copy of the patient information leaflet (PIL) and consent form will be retained by the participant and one will be filed in hospital records for reference. The original/hardcopies copy of consent forms will be stored in the study site file for consented patients; these will remain with the study team for the duration of the samples life which will then be archived once samples have been used up. Details of the study together with any suggested changes to clinical management emerging from it will be relayed to the participant's consultant and/or general practitioner (based on whether the patient is under primary or secondary care); consent to share this information with relevant healthcare professionals will be sought during the informed consent process. A recruitment target of one subject per month over the one year study period will allow the investigators to meet the study objectives and is within the resource capacity of the team.

7.2 Recruitment strategy

The recruitment phase will commence as soon as ethical and regulatory approval has been granted and the Sponsor green light has been given. Patient recruitment will be co-ordinated via the research team at the Leicester Diabetes Research Centre with support from divisions (2 and 5) of the Clinical Research Network for the East and West Midlands by setting up PIC sites from Primary care and Secondary care within the East and West Midlands area.

A team of five consultants in Upper Gastrointestinal (UGI) surgery provide bariatric surgery at the local population (Leicester /Leicestershire/ Rutland) in University Hospitals of Leicester NHS Trust. Over the last

10 years, more than 300 patients have been operated with RYGB within the University Hospitals of Leicester. This study will recruit from secondary care bariatric surgery clinics (Tier 4 follow-up clinics) at the University Hospitals of Leicester NHS Trust, University Hospitals of Birmingham and Luton and Dunstable University Hospital (Bedfordshire Hospitals NHS Trust), as well as from Private practice clinics (surgical clinics) and primary care (GP practices) for subjects who have been discharged from Tier 4 service.

Potential participants will be identified and/or contacted using direct and opportunistic marketing, using both verbal and written information about the research study, including invitation packs .If GP practices are willing to add notes and/or reminders to patient records within SystemOne to facilitate recruitment, then the study team will work with the CRN and GP practices to add notes and/or set-up reminders on eligible patient records for the GP to inform about the study during routine appointments.

Recruitment will be co-ordinated via the research team based at the Leicester Diabetes Research Centre. Recruitment will also be co-ordinated via CRN by setting up PIC sites from Primary care and Secondary care within the East and West Midlands area.

The following recruitment activities will be used:

1. Primary care
    a. GP practices (within the East & West Midlands area)

2. Secondary care
    a. Attendance at UHL Outpatients Clinics, for example:
        i. Bariatric Surgery follow-up clinics
        ii. Dietetic clinics
        iii. Obstructive Sleep apnoea clinics
        iv. Chemical Pathology clinics
    b. Secondary Care Databases – Database of patients who have undergone bariatric surgery over the last 10 years has been kept by the surgical team in Tier 4 service. This database will be used to approach eligible patients about the study.
    c. Discharged patients from Private Hospitals- use of patients who have been seen in private hospitals (e.g. Spire) but have been discharged from their care. Participant consultant will inform these discharged patients of the study and provide them with study material. Any

agreements required between the Consultant and Research Site will also be put in place before making contact with these patients.

d. NHS Sites outside of Leicester - where sites will be acting in the capacity of PIC sites to identify potential patients and carry out PIC activity.

3. Previous research participants

At the Leicester Diabetes Centre, around 50 adults who have undergone bariatric surgery have previously been screened for the "MOTION" study. We will recruit from this accessible pool of patients who are at least 1 year after RYGB if they have consented to take part In future research.

4. Key people in community/community events and meetings
    a. Identify and engage key people in the community including pharmacists, GP mentors, other Healthcare professionals and community workers to distribute both verbal and written information about the research study.

5. Recruitment/health fairs in community
    a. Participate in community events and open days to publicise the study and distribute information. This will consist of having a stand with all the study information and/or presenting the study at these events.

6. Study advertisement
    a. Distribute posters to publicise the study in primary and secondary care waiting rooms and within the community e.g. supermarkets, libraries, gyms and community centres.
    b. Advertise the study on social media (including Twitter, Facebook) as well as at the University Hospitals of Leicester NHS Trust and University of Leicester intranet which will include the study acronym and logo, a description about the study and contact details of the research team.
    c. Advertise the study on charity websites such as and not limiting to Obesity UK, which will include study poster, logo and brief description about the study and contact details of the research team.

7. Research Registry databases

a. Promoting study through Research registry databases such as the Leicester Research Registry to support study recruitment. This will include advertising of study material, a brief study description and contact details of the research team which will be circulated to Registry volunteers. The Study will also be promoted across Research Registry social media platforms.

7.3 Participant Flow

The outline of the patient flow through the study is highlighted in Figure 1 (page 18).

Visit 0 (Screening Visit, -14 days to –1 day), Visit 1 (Baseline Visit - 0 days), Visit 2 (7+5 days), Safety Call 1 [telephone call at day 14±5 (1 week after Visit 2)].

7.4 Informed Consent (Screening Visit, Visit 0, only)

Informed written consent will be obtained from all participants prior to any study procedures and only after they have had sufficient time (at least 24 hours) to read through the patient information sheet and ask any questions related to the study. The study clinician (or delegate) who has undergone consent training and holds an up-to-date Good Clinical Practice (GCP) certificate will obtain consent and this will be in the form of the participant's and researcher's dated signatures. The participant will be given a copy of the signed informed consent form along with a copy of the patient information sheet, a copy of informed consent will be retained in the medical records and the original will be retained in the Investigator Site File. Consent for ongoing participation will be checked at each study visit (and if applicable, following any updates to the PIS) and documented in the clinical notes and CRF by the study clinician.

7.5 Investigations

Primary Outcome Measurement

Glucose levels after mixed meal test (visits 1 and 2) – Nadir glucose levels

The primary outcome is the difference in nadir (lowest) glucose levels after the standardised MMTT between the two treatment options (additional NaCl 2g vs no additional NaCl).

For participants who will develop hypoglycaemia during the standardised MMTT (defined as blood glucose levels ≤3.0 mmol/l) the test will be stopped early for safety reasons, the glucose levels at the time that the test will be completed will be taken into account as the nadir glucose level.

For this, participants are required to fast for 10 hours before study visits 1 and 2, and prior to the measurement of glucose homeostasis at each visit, there will be a 24 hour standardisation period. The standardisation plans are described below:

24 Hour pre-visit Standardisation

For the 24 hours before study visits 1 and 2, participants will be asked to refrain from:

- completing any moderate to vigorous form of physical activity (see Appendix 1)
- consuming alcohol.

Participants will be asked whether or not they have refrained from the above in Visit 1 and Visit 2.

Mixed meal test standardisation

On the morning of study visits 1 and 2, participants will be provided with a standardised mixed meal test which will consist of 170mls of orange juice (Smooth Orange juice) followed by a pre-packed meal.

170 mls of smooth Orange juice will have an approximate % macronutrient content of:
Carbohydrates: 15.1g, Protein: 1.4g, Fat: 0g.

The pre-packed meal will have an approximate % macronutrient content of:
Carbohydrates: 61%, Fat: 26%, Protein: 9%, Salt: 0.15g
Overall the mixed meal test will consist of approximately (without additional 2g SALT)
Carbohydrates: 44g Protein: 7g, Fat:8g, Salt: 0.15g, Calories: 300kcal

Participants will be asked to consume the standardised MMTT over 10 minutes.
Glucose levels will be measured at the University Hospitals of Leicester NHS Trust pathology laboratories. Glucose will be measured in fasting conditions immediately before and at 7 time points after the standardised mixed meal test. Specifically, using cannulation of the participant's forearm, blood samples will be collected at baseline (i.e., pre-standardised mixed meal tolerance test) and then starting from the 'time of the last mouthful of the mixed meal tolerance test' at 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes. A total of 100 ml of blood (approximately 12.5 ml per sample) will be collected at visits 1 and 2. Blood for glucose levels will be collected into syringes and blood will be added to

Fluoride/Oxalate tubes immediately after collection. Samples will be transferred to the labs of the University Hospitals of Leicester for measurement of plasma glucose at the main hospital diagnostic laboratory.

Secondary outcomes

Biochemical Analysis (visits 1 and 2)

Blood for measurement of insulin, c-peptide and Glucagon Like Peptide-1 will be measured in fasting conditions immediately before and at 7 time points after the standardised mixed meal test - specifically at baseline (immediately before the standardised MMTT), at 15 minutes post-MMTT and then at 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes and 180 minutes post-MMTT to correspond with the glucose measurements.

Blood samples for insulin and c-peptide will be collected into pre-cooled blood collection tubes containing EDTA (EDTA 4.9 ml, 1 tube at each time point) to ensure sample viability for hormone/metabolite biochemical analysis. Blood collection tubes will be kept on ice prior to their use.

Blood samples for GLP-1 will be collected into pre-cooled blood collection tube (EDTA 4.9ml, 1 tube at each time point) containing 250microlitres aprotinin to ensure sample viability for hormone/metabolite biochemical analysis. Blood collection tubes will be kept on ice prior to their use.

After each blood withdrawal the cannula will be flushed with saline to maintain patency. Once blood samples have been collected they will immediately be spun in a refrigerated centrifuge (4°C) and the plasma will be obtained and aliquoted into Eppendorf tubes. These Eppendorf tubes will then be frozen (initially at -20°C but then transferred to -80°C on the same day as collection) until required for analysis. The GLP-1 samples will be transported to Loughborough University for subsequent analysis using dry ice. Samples for measurement of insulin and c-peptide will be analysed at Leicester General Hospital by the Research Scientists based at Leicester Diabetes Centre and then disposed of in accordance with the Human Tissue Authority's Code of Practice.

Following sample analysis, where a participant has consented, plasma samples will be stored indefinitely for future research use. A copy of the consent form will be retained for the duration of the samples life to ensure that they will be used following the conditions that participants have given consent for.

Study Questionnaires

The participant will be asked to complete the following validated questionnaires (see Appendices 2 and 3):

Edinburgh Hypoglycaemia Scale (EHS) – this will assess the symptoms of hypoglycaemia during screening (Visit 0) as well as during the mixed meal tolerance test at visits 1 and 2 (time points 0'(before initiation of the MMTT), 15'(after consumption of the last mouthful of the meal), 30', 60', 90', 120', 150', 180').This will be completed at the time-points above once blood samples have been drawn.

Sigstad dumping score – This will assess symptoms of dumping during the mixed meal tolerance test at visits 1 and 2 (time points 0' (before initiation of the MMTT), 15' (after consumption of the last mouthful of the meal), 30', 60', 90', 120', 150', 180'). This will be completed at time-points above once blood samples have been drawn.

Other Measurements and Data Collection

Anthropometric Measures (visits 1, 2)

Body weight will be measured to the nearest 0.1kg, body fat percentage will be measured to the nearest 1% and muscle mass will be recorded to the nearest 0.1kg using bioelectrical impedance equipment (i.e. Tanita™ scales) while the participant is wearing no shoes and socks and after the removal of any heavy items of clothing.

Height will be measured using a portable stadiometer to the nearest 0.5cm with the participant wearing no shoes.

Blood pressure and pulse rate will be measured using an automated sphygmomanometer for the arm whilst the patient is seated after resting quietly for five minutes. Three measurements will be obtained for blood pressure and pulse rate and an average of the last two will be used.

Demographics (Visit 0)

The date of birth, gender, race, smoking and drinking habits will be recorded at the familiarisation visit (visit 0).

Medical History (Visit 0)

Details of any history of disease or surgical interventions will be recorded at the familiarisation visit (visit 0).

Physical Examination (Visit 0)

A general physical examination will be performed at the familiarisation visit (visit 0).

7.6 Randomisation (Visit 1)

Randomisation will take place at the level of the individual using an independent online computerised randomisation service (sealed.envelope.com).

Eligible participants will be randomly assigned to one of the Groups as per below in a 1:1 ratio to the following treatment sequence:

(Group 1) MMTT with additional 2g table salt, followed by MMTT without additional table salt after a "washout" period of 7 days or

(Group 2) MMTT without additional 2g table salt, followed by MMTT with additional 2g table salt after a "washout" period of 7 days.

Participants will be informed of their randomisation assignment during the Baseline Visit. Letters will also be sent out to the participants GP which will notify them of their patient's participation in the study and inform them of study results.

7.7 Subsequent Assessments

Table 1 (pages 32-33) shows the visit numbers and window periods for the visits.

Visit 0 (Screening Visit) – clinic visit (- 14 days to - 1 day)

During this visit, there will be an eligibility check of inclusion/exclusion criteria, past medical and surgical history, demographics, concomitant medications recorded and a safety assessment including physical examination and a urine pregnancy test for females of child bearing potential will be performed. Screening blood tests will also be performed; these will be taken for HbA1c, full blood count (FBC), renal function (U+Es and eGFR) and Liver function (LFTs).

Confirmation of eligibility will take place once all blood results have been received and reviewed by a delegated clinician. Participants will be telephoned by a member of the research team to confirm whether or not they are eligible to continue in the study. If eligible, Visits 1 and 2 will be arranged. If not eligible, participants will be thanked for their interest in the study.

Visit 1 (Baseline Visit) – clinic visit (day 0)

Participants will arrive in fasted condition (overnight fast, not eating/drinking anything except of water since

23:00 at previous night) and having not consumed alcohol or performed moderate to vigorous exercise in the 24 hours prior to this visit. During this visit, there will be a check for ongoing consent to participate in the study, record of concomitant medications and randomisation. Anthropometrics (including body fat percentage-Tanita), BP and pulse rate will be measured and participants will be asked regarding AE/SAE. All female participants of child bearing potential will have a repeat urine pregnancy test. A cannula will be inserted for blood sampling. A 3-h MMTT with or without additional 2g NaCl (NaCl will be diluted with orange juice, which is part of the MMTT) based on treatment sequence will take place). Patients will be allowed to consume up to 150mls of water after the first 30mins after the ingestion of the MMTT. Blood sampling for measurement of plasma glucose, c-peptide, insulin and GLP-1 will be performed at fasting state (immediately before the MMTT) and at 15', 30', 60', 90', 120', 150' and 180' after ingestion of MMTT. Blood glucose levels will also be measured through a glucose meter at the same time points. An extra capillary blood glucose test will be performed outside the pre-specified time points for blood sampling if participants report new onset/ worsening of neuroglycopenic or autonomic/malaise symptoms suggestive of hypoglycaemia after the first 60min since MMTT consumption (see also Appendix 3). Hypoglycaemia will be treated (and mixed meal tolerance test will be completed) in accordance with the Hypoglycaemia Guideline for Adults of the University Hospitals of Leicester, if CBG/blood glucose levels are ≤3.0 mmol/l with or without symptoms of hypoglycaemia [if CBG has been performed (test outside pre-specified time points) and patient clinically well, blood sampling will be performed for plasma glucose and rest of biochemical markers immediately before treatment of hypoglycaemia]. During the mixed meal test, patients will complete validated questionnaires on dumping symptoms (38), as well as on hypoglycaemia symptoms (39). Severe neuroglycopenic symptoms (defined as ≥5 in Edinburgh Hypoglycaemia Scale in at least one of these symptoms) in participants with glucose levels between 3.1 mmol/l and 3.9 mmol/l will be treated in accordance with the Hypoglycaemia Guideline for Adults of the UHL and the MMTT will be completed. Those participants with glucose levels ≥3.1 mmol/l who decide to stop the MMTT due to severe neuroglycopenic symptoms will be excluded from the study and subsequently from the analysis

Visit 2 – clinic visit (day 7+5 days)

Participants will also arrive in fasted condition (overnight fast, not eating/drinking anything except of water since 23:00 at previous night)) and having not consumed alcohol or performed moderate to vigorous exercise in the 24 hours prior to visit 2. During this visit, there will be a check for ongoing consent to participate in the study and record of concomitant medications. Anthropometrics (including body fat percentage-Tanita), BP and pulse rate will be measured and participants will be asked regarding AE/SAE over last 1 week. A cannula

will be inserted for blood sampling. A 3-h MMTT with or without additional 2g NaCl (NaCl will be diluted with orange juice, which is part of the MMTT) based on treatment sequence (opposite to what participants received at the visit 1) will take place. Patients will be allowed to consume up to 150mls of water after the first 30mins after the ingestion of the MMTT. An extra capillary blood glucose test will be performed (outside the prespecified time points for blood sampling), if participants report new onset/ worsening of symptoms suggestive of hypoglycaemia (autonomic, malaise or neuroglycopenic, see also Appendix 3) after the first 60min since MMTT consumption. Hypoglycaemia will be treated (and mixed meal test will be completed) in accordance with the Hypoglycaemia Guideline for Adults of the University Hospitals of Leicester, if CBG/blood glucose levels are ≤3.0 mmol/l with or without symptoms of hypoglycaemia [if CBG has been performed (test outside pre-specified time points) and patient clinically well, blood sampling will be performed for plasma glucose and rest of biochemical markers immediately before treatment of hypoglycaemia]. During the mixed meal test, patients will complete validated questionnaires on dumping symptoms, as well as on hypoglycaemia symptoms. Severe neuroglycopenic symptoms (defined as ≥5 in Edinburgh Hypoglycaemia Scale in at least one of these symptoms) in participants with glucose levels between 3.1 mmol/l and 3.9 mmol/l will be treated in accordance with the Hypoglycaemia Guideline for Adults of the UHL and the MMTT will be completed. Those participants with glucose levels ≥3.1 mmol/l who completed early the MMTT due to severe neuroglycopenic symptoms will be excluded from the study and subsequently from the analysis.

Safety Call 1 – Telephone call (day 14±5 days) – End of the study

Participants will be contacted by telephone within 7 days of completion of the 2nd treatment period (Visit 2) in order to capture any potential Adverse Events.

7.8 Definition of End of Trial

The end of trial is the date when analysis of study data is complete.

7.9 Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time without needing to give a reason. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with study requirements

- An adverse event which requires discontinuation of the study procedures (such as failure of cannula insertion) or results in inability to continue to comply with study procedures
- Consent withdrawn
- Early termination of standardised MMTT because of severe neuroglycopenic symptoms/ severe autonomic symptoms despite that blood glucose levels/CBG are >3.0 mmol/l.
- Lost to follow up

The reason for withdrawal will be recorded in the CRF and medical records if known. If the participant is withdrawn due to an adverse event, the investigator will arrange for safety follow-up visits or telephone calls until the adverse event has resolved or stabilised. The duration of safety follow-ups will be up to 1 week after the end of the study.

If a participant is withdrawn from the study for whatever reason, data and samples collected up to that point will be used unless participant states otherwise. However, it is noted that the primary analysis for the study will be on a complete cases basis (i.e. participants who attend both mixed meal tolerance tests).

Any decisions regarding the withdrawal of participants from the study will be made by the study clinician and the Chief Investigator.

Each participant will have a copy of the consent form and patient information leaflet placed in their hospital medical records. A standard label will be used on the front of the medical notes to highlight to any reviewer that this individual is taking part in the study and any issue regarding contra-indication of a procedure or medication outside of the study should be discussed with a study clinician.
The standard label template is provided by the local trust and should contain the following information:

PATIENT TAKING PART IN A CLINICAL TRIAL

Study Name.

Patient ID No. . . . . .

Investigator: . . . . . .

Telephone: . . . . . .

Date Consent Form Signed . . . . . .

TRIAL START <date> TRIAL FINISH <date>

Don't destory the records before <date>

7.10 Source Data

Source documents are original documents, data, and records from which participants' CRF data will be obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication will be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). All documents will be stored safely in a secure office environment. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code only, and not by name.

Table 1. Study procedures and data collection during the study.

	Key time points			
Visits (No)	Screening – Visit 0	Baseline – Visit 1	Visit 2	Safety call 1*
	-14 days to -1 day	0 days	7 (+5) days	14(±5) days
Study procedures				
Inclusion/exclusion criteria	X	X#	X#	
Informed consent	X			
Randomisation		X		
Clinician/Nurse appointment	X	X	X	X
Cannula insertion		X	X	
3-h mixed meal tolerance test (with or without added 2g NaCl, as per randomization)		X	X	
Blood sampling	X(1)	X(8)	X(8)	
Blood glucose monitoring		X(8)	X(8)	
Filling CRF forms	X	X	X	X
Centrifuge of samples		X(8)	X(8)	
Storing of samples		X(8)	X(8)	
Labelling of samples		X	X	

Physical Examination	X			
Data collection				
Subject demography	X			
Medical/Surgical history	X	X	X	
Prior medication history	X			
Concomitant medication	X			
Weight [Including body fat % (Tanita)]		X	X	
Height		X	X	
Visits (No)	Screening – Visit 0	Baseline – Visit 1	Visit 2	Safety call 1*
	-14 days to -1 day	0 days	7 days (+5) days	14(±5) days
Study procedures				
Blood Pressure	X	X	X	
Pulse Rate	X	X	X	
Renal function test	X			
Liver function test	X			
Haematology profile	X			
HbA1C	X			
Urine pregnancy test	X*	X*	X*	
Change in concomitant medication and diseases		X	X	
Hypoglycaemia questionnaire		X(8)	X(8)	
Dumping questionnaire		X(8)	X(8)	
Physical Activity and alcohol question (last 24 hours)		X	X	
AE/SAE recording (including hypoglycaemia)		X	X	X

X#: Check for any changes in patients exclusion criteria

X*: For all female patients of childbearing potential.

8. TREATMENT OF STUDY PARTICIPANTS

8.1 Description of Study Treatment

The study nutritional supplement is table Salt (NaCl). The dosage will be 2g and the NaCl will be diluted in 170mls of orange juice which will be part of the MMTT. Two (2) gram of NaCl corresponds to the 33% of the recommended daily NaCl intake by the British Heart Foundation.

All potential contra-indications to use of NaCl will be checked as part of the eligibility criteria.

Study nutritional supplement may be suspended or stopped by the study team if they become aware of the participant undergoing any contra-indicated procedure out of study clinical care. This will be documented in the individual's study and medical notes and decision on any potential withdrawal from the study on this basis will reside with the chief investigator.

8.2 Storage and dispense of Study Treatment

The study nutritional supplement (table Salt, NaCl) will be stored at room temperature in a designated, restricted area for medication storage at Leicester Diabetes Centre, Leicester General Hospital. The study nutritional supplement will be provided to the study participants by the nursing team or doctors of the study at Leicester Diabetes Centre, Leicester General Hospital, in accordance with the study protocol. More specifically, the NaCl will be diluted in 170mls of orange juice and will be consumed by the participant as part of the MMTT. A high precision (0.01g) digital scale will be used for accurate measurement of NaCl 2g.

8.3 Concomitant Medication

Participants are allowed to continue the use of concomitant medication, which will be recorded in the CRF. If concomitant treatment has to be changed during the study period, this must be reported on the CRF provided (trade name and/or generic name) and in the participant's medical records. Addition of any medication that will significantly influence weight and glucose levels, such as systemic corticosteroids (oral or injectable), during the period of the study, is reason for discontinuation of the participant in the study.

9. SAFETY REPORTING

9.1 Definitions

Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participant, which does not necessarily have to have a causal relationship with this treatment (the study nutritional supplement).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study nutritional supplement, whether or not considered related to the study nutritional supplement.

Adverse Reaction (AR)

All untoward and unintended responses to the nutritional supplement of the study related to any dose. The phrase "responses to the nutritional supplement of the study" means that a causal relationship between the study nutritional supplement and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the study nutritional supplement qualify as adverse reactions. There are no expected AR in this study

Severe Adverse Events

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Serious Adverse Event or Serious Adverse Reaction (SAE or SAR)

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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 inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Expected Serious Adverse Events/Reactions (SAE)

This study is using a nutritional supplement (NaCl) at a dose in accordance with the recommendations of British Heart Foundation and British National Formulary. Therefore, no SAE/Rs are expected.

Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information

This study is using a nutritional supplement (NaCl) at a dose in accordance with the recommendations of British Heart Foundation and British National Formulary. Therefore, no SUSARs are expected.

9.2 Definition of hypoglycaemia

Definition of hypoglycaemia during the mixed meal tolerance test

Hypoglycaemia during the mixed meal test will be defined as plasma glucose ≤ 3.0 mmol/l. It is noted that decisions regarding treatment of hypoglycaemia during the MMTT will be made in accordance with venous blood glucose/capillary blood glucose values and symptoms of the patient (instead of plasma glucose levels which will be performed at the UHL lab). More specifically if venous blood glucose/capillary blood glucose levels are ≤3.0 mmol/l independent of symptoms, the MMTT will be stopped and the patient will receive treatment for hypoglycaemia in accordance with Hypoglycaemia Guideline for Adults of the University Hospitals of Leicester. [If CBG has been performed (test outside pre-specified time points) and patient clinically well, blood sampling will be attempted for plasma glucose and rest of biochemical markers immediately before treatment of hypoglycaemia]. If the participant is asymptomatic or having autonomic/other symptoms (in accordance with Appendix 3) will receive glucose from the oral route (15-20g), whilst if the participant is having neuroglycopenic symptoms, then treatment will be with IV dextrose. It is noted that it is not expected that patients will experience severe hypoglycaemia during the study as patients will be excluded at screening if they have an established diagnosis of postprandial hyperinsulinaemic hypoglycaemia after RYGB.

9.3 Reporting Procedures for All Adverse Events

All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study nutritional supplement, will be recorded on the CRF and medical records.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study nutritional supplement, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study nutritional supplement as judged by a medically qualified investigator or the Sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs. The duration of safety follow-ups will be at least 7 days after ingestion of nutritional supplement.

It will be the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment.

A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable. The duration of safety follow-ups will be at least 7 days after ingestion of nutritional supplement.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

The relationship of AEs to the nutritional supplement will be assessed by a medically qualified investigator.

9.4 Reporting Procedures for Serious Adverse Events

All SAEs will be reported to the Sponsor immediately. The SAE will be reported using appropriate forms and according to the Sponsor SOP for reporting serious adverse events, Additional information can be provided if requested to the Sponsor and main Research Ethics Committee (REC) (e.g. in the event of a death). The Principal Investigator or another delegated physician is responsible for the review and sign off of the SAE and the assessment of causality and expectedness.

The Sponsor will perform an initial check of the information and ensure that it is reviewed at the next R&D management meeting. All SAE information must be recorded on an SAE form and sent to the Sponsor. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form and sent to the Sponsor.

The Sponsor will report all SUSARs to the REC concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The Sponsor and the Chief Investigator/Principal Investigator or delegated medic will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

In addition to the expedited reporting above, the Chief Investigator will submit once a year throughout the clinical study or on request an 'Annual Report' to the REC which lists all SAEs/SUSARs that have occurred

during the preceding 12 months. The CI will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

The investigator site file will contain documentation for:-

- SAE, SAR and SUSAR reports
- Evidence of submission of SAEs to the Sponsor within 24 hours of the team becoming aware of an event
- Evidence of timely SUSAR submission to the main REC

9.5 Safety issues

We do not foresee any adverse events over and above those associated with everyday life and routine health care that could be attributable to the study. The study involves only ingestion of a mixed meal tolerance test with and without added 2g table Salt (NaCl) and venepuncture for cannula insertion at 2 visits to withdraw venous blood at multiple time points. Hence, the study carries a very low risk of having untoward effects. However, all participants will undergo cannula insertion which can occasionally result in bruising, swelling and temporary discomfort. Any safety event occur it shall be reported according the process described above. Hypoglycaemia during the mixed meal tolerance test will be also treated as described above.

In the case of clinically relevant readings being collected (e.g. blood pressure 170/95 etc.), a letter will be sent to the GP on the same day stating the problem and recommending a review of the condition/or repeating the test. Emergencies (e.g. BP >220/120 at any visit or K+ >7.0 during screening visit) will be referred by a doctor to the appropriate services depending on the nature and history of the condition.

10. STATISTICS

10.1 Description of Statistical Methods

Descriptive statistics will be calculated to outline the characteristics of the study sample. The demographic and clinical characteristics will be tabulated and summarised by treatment group and in total. Normality of data will be assessed using histograms and box plots. Depending on the distribution of data, participant characteristics will be reported as mean (SD) or median (IQR) and number (percentage) for continuous variables and for categorical variables respectively.

The primary outcome is the difference in nadir (lowest) glucose levels between the two treatment options (2g additional NaCl vs no additional NaCl) after the standardised mixed meal test for two period crossover design. Data will be collected at time points (minutes) 0, 15, 30, 60, 90, 120, 150, 180. The AUC [(Area Under the Curve) calculated using trapezium rule] will be used for summarising the response to the 2g additional NaCl with the standardized mixed meal tolerance test. Paired t test or equivalent non-parametric tests will be used to analyse the AUC and the difference in nadir glucose levels. The primary and main secondary outcomes will be analysed on a complete case basis. In instances of hypoglycaemia (glucose levels ≤3.0 mmol/l) when the MMTT will be terminated early, the last data point will be carried forward for comparison of AUC values with and without treatment. The difference between groups will be assessed by treatment x type of surgery interaction.

The assumptions associated with each model will be assessed and where these are not met alternative models or parameterisations will be considered. As this study design incorporates one week washout, it will be assumed that issues sometimes associated with crossover trials of time and period effects will not be an issue here.

A value of $p<0.05$ will be considered statistically significant for all analyses. Statistical analyses of the baseline data and all future analysis will be carried out using STATA version 15."

10.2 The Number of Participants

The primary outcome will be nadir glucose levels after a MMTT. This is a proof-of-concept study and there are no previous data available on the effect of sodium intake on glucose homeostasis after RYGB in humans. Previous PPH pilot studies have recruited up to 9 patients (20, 21) and demonstrated that an increase in nadir glucose levels by 0.6 mmol/l may be associated with a clinically meaningful reduction in postprandial hypoglycaemia. Assuming a standard paired test the proposed research was powered (80%) to detect a 0.6mmol/l difference in the primary outcome of nadir (lowest) glucose between the interventions with alpha

set at 5% and within person correlation of 0.05 assuming a standard deviation of 0.45mmol/l. On this basis, 14 participants after RYGB are required for this crossover study, considering a 20% drop-out rate. Low dropout rate is expected due to short duration of the study (3 visits, 8 days treatment period).

10.3 The Level of Statistical Significance

All statistical tests will be two-sided and significance will be tested at the 5% level.

11. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised and delegated members of the study team, authorised representatives from the sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

12. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures (SOPs).

The University of Leicester, as Sponsor, operates a risk based monitoring and audit program, to which this study will be subject.

The study team will conduct regular QC checks on study data to ensure all data that is captured is accurate for the duration of the study. The study team will also be responsible in ensuring the site files are maintained and all relevant study documents are within the site file.

All source data, study documents, and participant notes will be made available for monitoring, audits and inspections by the Ethics Committee, the Sponsor and the Regulatory Authority.

13. CODES OF PRACTICE AND REGULATIONS

13.1 Ethics

Participants will be free to withdraw at any time from the study without giving a reason and without their legal rights being affected. All study procedures including risks involved will be explained clearly to the participant at the Screening Visit and subsequently before each procedure is performed.

The overall care and comfort of the participant will be considered paramount at all times during the study.

13.2 Sponsor Standard Operating Procedures

All relevant Sponsor SOPs will be followed to ensure that this study complies with all relevant legislation and guidelines. Where applicable, host and LDC SOPs will be followed.

13.3 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

13.4 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

13.5 Approvals

Once Sponsor (University of Leicester) authorisation has been confirmed, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities [Health Regulation Authorities (HRA) in the UK], and host institution's research and development department for written approval. The study will not commence until Sponsor green light is given.

Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial and non-substantial amendments to the original approved documents.

13.6 Participant Confidentiality

The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so. Participants will be identified only by their initials and unique study ID number on the CRF and the electronic database (REDCAP). Samples will only be identified Study ID number, visit number and time of collection. Access to the database, samples and all documents will be restricted to study staff and authorised personnel from the Sponsor, host NHS Trust and regulatory authorities. The database will be maintained and accessed via University Hospitals of Leicester networks and servers. Study data will be stored for 5 years.

All research data will be kept in a secure office environment within Leicester Diabetes Centre, Leicester General Hospital during the active phase of the study and until the data have been analysed. Consent forms will remain on site at all times during the life-cycle of the sample, it will then be archived in line with University of Leicester policy unless consent has been given for the samples to be used for future ethically approved research. In such instances, the consent form will be retained for evidence of the provenance of the sample and the consent from may be transferred to another organisation/individual where material transfer agreements are in place. The consent form will be retained by the custodian of the sample(s) at all times. Where consent has been given, identifiable data will be retained on a volunteer's database for future research.

14. DATA HANDLING AND RECORD KEEPING

All study documentation will be managed in accordance with ICH-GCP, the UK Policy Framework for Health and Social Care Research (2017), General Data Protection Regulation (2018) and the Data Protection Act (2018).

Participants will be allocated a unique Study ID number which will be used on all research documentation from the point of consent onwards.

The Case Report Forms (CRFs) and research database (REDCAP) will identify participants by their Study ID number, and samples will contain the Study number in addition to the visit number and time of collection. As such, all source data and samples will be pseudonymised and access will be limited to the delegated members of the research team (including those conducting analysis of samples working at Loughborough University). Access to the source data, database and Trial Master File will also be granted to delegated individuals from the Sponsor (University of Leicester), the host NHS organisation (University of Leicester), the host NHS organisation (University of Leicester NHS Trust) and regulatory authorities for monitoring and auditing purposes.

Database containing identifiable information for the purpose of contacting participants will be held on the host NHS organisation (University of Leicester NHS Trust) servers, and access will be limited to delegated members of the research team only. The Study database (REDCAP) is password protected and is owned and maintained by the host NHS organisation (University of Leicester NHS Trust). Final data analysis will be conducted on severs owned and maintained by the host NHS organisation (University of Leicester NHS Trust) and /or the Sponsor (University of Leicester) and/or those delegated the task of sample analysis (Loughborough University).

The Trial Master file and CRFs will be retained in a secure location within the Leicester Diabetes Centre and will then be archived for 5 Years following the end of study in line with the Sponsor SOP.

15. STUDY GOVERNANCE The study will be conducted in accordance with the Research Governance Framework for Health and Social Care Research, ICH GCP and the Data Protection Act. The Sponsor responsible for checking research governance arrangements will be the University of Leicester.

15.1 Trial Steering Committee (TSC)

There will be no trial steering committee for this study. Regular meeting will be conducted between the study team, the chief investigator and the co-investigators to discuss study progress.

15.2 Data safety monitoring committee (DSMC)

There will be no data safety committee for this study. All data safety matters will be reviewed on a regular basis by the study team, the chief investigator and the co-investigators.

15.3 Operational Meetings (TMG)

Operational meetings will include the chief investigator, other senior investigators and the day-to-day project management team. The group will meet monthly or bi-monthly depending on need, either face-to-face or by teleconference, to discuss the details and logistics of recruitment, retention and follow-up data collection and participant safety.

16. PUBLICATION POLICY

Any original findings will be published as conference abstracts or as papers in reputable refereed journals. Authorship will include those listed as investigators in the research protocol. These individuals will take personal responsibility for their identified area of expertise and will identify their contributions to the research process in any publication. Collaborators, other contributors, funding bodies, the Sponsor and the authorizing REC will be acknowledged.

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APPENDIX 1 – Recall question regarding physical activity and Alcohol over the last 24hours.

"In the last 24 hours have you undertaken any activities that have noticeably accelerated your heart rate and made you breathe harder than normal?" - Yes/No.

“In the last 24 hours have you consumed any Alcohol?”- Yes/No

APPENDIX 2– Dumping questionnaire

Sigstad's scoring system for diagnosis of dumping syndrome

Symptoms	Grade
Shock	+5
Fainting, syncope, unconsciousness	+4
Desire to lie or sit down	+4
Breathlessness, dyspnoea	+3
Weakness, exhaustion	+3
Sleepiness, drowsiness, apathy, falling asleep	+3
Palpitation	+3
Restlessness	+2
Dizziness	+2
Headaches	+1
Feeling of warmth, sweating, pallor, clammy skin	+1
Nausea	+1
Abdominal fullness, meteorism	+1
Borborygmus	+1
Eructation	−1
Vomiting	−4

APPENDIX 3 – Edinburgh Hypoglycaemia Symptom Scale

	1	2	3	4	5	6	7
	No trouble at all	Minor trouble	Mild trouble	Moderate trouble	Quite severe trouble	Severe trouble	Very severe trouble
Autonomic symptoms							
Sweating							
Palpitations							
Shaking							
Hunger							
Neuroglycopenic symptoms							
Confusion							
Drowsiness							
Odd Behavior							
Speech Difficulty							
Incoordination							
Malaise symptoms							
Nausea							
Headache							