The impact of a fat-rich preload before a carbohydrate-rich meal on glucose homeostasis in patients without diabetes after sleeve gastrectomy (the CARLOTA study): A proof-of-concept, randomised, open-label, crossover study.

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AMENDMENT HISTORY

Amendment number	Protocol version number	Details of changes made
NSA 1	V1.1	Amendment to change recruitment strategy listed within the protocol, including the use of research registries, databases and charities such as Obesity UK to target a wider population.
NSA 21	V2.0	Protocol amendment to change some of the inclusion/exclusion criteria (diabetes status, liver cirrhosis, steroid use and lactose intolerance). Removal of TMG's and addition of Ops meetings.
NSA 3	2.1	Within this amendment, we have updated the recruitment strategy within the current approved protocol to include the use of PIC sites (i.e NHS secondary care and primary care sites) within the East and West Midlands. We also intend to collaborate with Consultants who are contracted to private hospitals but are registered with the ICO as Data Controllers and will therefore act as an independent party to recruit discharged patients from these private hospitals. Minor change to protocol amendment table to correct Substantial amendment 2 to NSA 2.
NSA 4	2.2	Within this amendment we have updated the study exclusion criteria, to provide clarity that those who have taken part in a CTIMP less than one month will be excluded. We have also extended the study recruitment end date to April 2023. Within this amendment we will also include University Hospitals of Birmingham to be set up as a PIC site. Study documents such as patient thank you letter updated to reflect new recruitment end date.

NSA05	V2.3	Within this amendment, we have updated the study exclusion criteria as we have removed the following exclusion criterion "People who are on regular painkillers (codeine phosphate, paracetamol, morphine or NSAIDS)". However, we are not amending the following statement "Participants will also be asked to refrain from consuming paracetamol 48 hours before study visits." We have updated the recruitment section in line with a change of name for one of the PIC sites from Derby Hospitals Foundation Trust to University Hospitals of Derby and Burton NHS Foundation Trust. We have also extended the study end date to 30.09.2023. The patient thank you letter document has also been updated to reflect the new study end date.
NSA06	V2.4	Within this amendment we have extended the recruitment end date to 31.10.2023. The overall study end date has been extended to 31.01.2024 to allow time to complete sample and statistical analysis. The patient thank you letter document has also been updated to reflect the new overall study end date.

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
PIL	Participant/ Patient Information Leaflet
PHH	Postprandial Hyperinsulinaemic Hypoglycaemia
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SG	Sleeve Gastrectomy
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 sleeve gastrectomy (SG), the most commonly performed bariatric procedure worldwide. The treatment options for PHH are limited. The underlying pathophysiology of PHH after SG is unclear, however rapid gastric emptying and the rapid nutrient delivery at the distal small intestine postoperatively seem to have a major impact on the glucose homeostasis after SG and to be important contributing factors to PHH.

Emerging evidence suggests that consumption of virtually carb-free, fat-rich preloads (20-30 minutes before a carbohydrate-rich meal) delays the gastric emptying of the carbohydrate-rich meal leading to a more gradual absorption of glucose and subsequently to reduced postprandial glucose and insulin levels in patients with and without diabetes who have not undergone bariatric surgery (BS).

So, if a fat-rich preload before a carbohydrate-rich meal could also delay the gastric emptying after SG and lead to a subsequent reduction of peak glucose and post-meal insulin levels, then a fat-rich preload could be an alternative treatment option for PHH after SG for people who cannot tolerate a low carbohydrate diet.

Objective of the study: To investigate the effect of a fat-rich preload, consumed 30 minutes before a carbohydrate-rich meal, on postprandial glucose homeostasis and gastric emptying in patients without diabetes (including those with type 2 diabetes in remission after SG.

Aim of the study: The aim of study is to investigate whether a fat-rich preload, consumed 30 minutes before a carbohydrate-rich meal, could be a potential treatment option for PHH after SG.

Hypothesis: We hypothesize that a fat-rich preload, consumed 30 minutes before a carbohydrate-rich meal, in patients without diabetes after SG, will lead to higher postprandial nadir glucose levels by reducing peak postprandial glucose levels and postprandial insulin secretion due to a reduced rate of gastric emptying.

Duration of the study: 12 months

Design of the study: A proof of concept, open-label, crossover study. Eligible subjects without diabetes (including those with type 2 diabetes in remission) after SG (n=12) will be randomised to one of the following treatment sequences: a) standardised mixed meal tolerance test (MMTT) [plus 1g dispersible paracetamol] with a fat-rich preload (28g of nuts) 30 minutes before the MMTT

followed by (at least 1 week later) a MMTT (plus 1g dispersible paracetamol) without a fat-rich preload or b) vice versa.

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 (plus 1g dispersible paracetamol) with or without a fat-rich preload consumed 30 minutes before the mixed meal, based on treatment sequence. The fat-rich preload will consist of 28g of nuts and will be consumed over 10 minutes with 80mls of water. The standardised mixed meal will consist of a liquid oral supplement (Nutricia Fortisip Milkshake, 220mls, 330kcal, 12.76g fat, 40.5g carbohydrates, 13.2g protein) and will also be consumed over 10 minutes. Twenty ml of water with the dispersible 1g of paracetamol will be added to the mixed meal.

Blood samples include glucose, insulin, GLP-1 and paracetamol levels at the following time points: -30' (immediately before the preload consumption), -15' and 0' minutes (immediately before the meal consumption) and 15', 30', 45', 60', 90', 120', 150', 180' minutes after the meal consumption. Moreover, validated questionnaires regarding dumping symptoms and hypoglycaemia symptoms will be completed at the same time points as blood sample collection immediately after the blood sample has been obtained.

After a 7-day washout period, participants will undergo a second standardised MMTT plus 1g of dispersible paracetamol [Visit 2, day 7 (+7) of the study] with or without a fat-rich preload (opposite to first treatment option) and procedures will be repeated as described in Visit 1.

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% suffer from severe and complex obesity (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).

Sleeve gastrectomy (SG) is the most commonly performed bariatric surgery procedure worldwide (5). Despite successful weight loss and weight maintenance, some long-term complications can develop after SG, such as nutritional and vitamin deficiencies (6), early dumping syndrome (7-8) and postprandial hyperinsulinaemic hypoglycaemia (PHH) (9-12).

Postprandial hyperinsulinaemic hypoglycaemia (PHH) is a condition characterized by hypoglycaemic symptoms occurring 1 to 3 hours after a meal accompanied by a low venous glucose value, typically preceded by a high rise in both glucose and insulin concentrations (13-14). PHH has been described since the 1940s as a complication of gastric resection in patients suffering from peptic ulcers and was named “late dumping”. This condition warrants further attention due to the increased number of bariatric procedures performed worldwide in recent years. Recurrent PHH after bariatric surgery is associated with reduced quality of life, reduced functional ability (inability to work, drive, care for others) and weight regain (15,16). In addition, an increased rate of accidental deaths, syncopal episodes and seizures among patients who have undergone bariatric surgery has been reported, and it is speculated that this could be due to neuroglycopenic symptoms as result of severe PHH (17,18).

The incidence of hypoglycaemia during daily life ranges between 14-33% after SG, depending on the definition of hypoglycaemia, the population studied and the diagnostic assessment tool used (9-12). Risk factors for developing symptoms of postprandial hypoglycaemia after SG include younger age, no history of diabetes preoperatively and lower BMI preoperatively (12,19-21).

Treatment options for PHH after SG are limited and patients are typically encouraged to follow dietary modifications consisting of small, frequent meals which are low in carbohydrate. Although potentially efficient, a low carbohydrate diet presents large compliance problems and may not be applicable to all patients suffering from PHH (22). Medical treatments include mainly acarbose and somatostatin analogues (23-25), but their limited effectiveness, side effects and cost limit their use (22). Thus, alternative treatments are needed; however the development of effective treatments for PHH after BS requires a detailed understanding of the underlying mechanisms of the condition.

Currently, the underlying pathophysiology of PHH after SG is incompletely understood, but the most likely explanation is PHH it is a physiological result of rapid gastric emptying and subsequent

rapid glucose absorption at the distal small bowel postoperatively (26-28). The rapid arrival and rapid absorption of undigested carbohydrates at the distal small bowel leads to a prominent early spike in postprandial glucose levels which subsequently triggers a corresponding over-secretion of insulin and gut hormones such as Glucagon Like Peptide-1 (GLP-1), leading to PHH (21,28-30). Indeed, people who experience PHH after SG have increased endogenous insulin secretion compared to those without PHH (21).

Emerging evidence suggests that the consumption of virtually carb-free, fat-rich preloads (20-30 minutes before a carbohydrate-rich meal) delays the gastric emptying of the carbohydrate-rich meal. This leads to a more gradual absorption of glucose and subsequently reduces postprandial glucose and insulin levels in patients with and without diabetes who have not undergone bariatric surgery (31-34). So, if a fat-rich preload before a carbohydrate-rich meal delays the gastric emptying after SG and leads to a subsequent reduction in peak glucose and post-meal insulin levels, then a fat-rich preload could be an alternative treatment option for PHH after SG for people who cannot tolerate low carbohydrate diets.

On the other hand, consumption of virtually carb-free, protein-rich preloads have strong dose-dependent insulinotropic effects likely due to both direct and incretin-mediated interactions of protein and amino acids with β cells (31). So, protein-rich preloads before a carbohydrate-rich meal after SG may not be the ideal option, as it is likely that they will lead to increased peak insulin levels after the meal and may subsequently increase the risk for PHH postoperatively.

This study will be the first to investigate the effect of a fat-rich preload 30 minutes before the consumption of a standardised carbohydrate rich meal on glucose homeostasis in subjects without diabetes after SG.

Research Hypothesis: A fat-rich preload, consumed 30 minutes before a carbohydrate-rich meal, in patients without diabetes after SG, will increase the lowest postprandial glucose levels by reducing the peak postprandial glucose levels and peak postprandial insulin secretion due to a reduced rate of gastric emptying.

4. OBJECTIVES

The aim of the study is to investigate the effect of a fat-rich preload, consumed 30 minutes before a carbohydrate-rich meal on glucose, insulin and gut hormones in patients without diabetes (including those with type 2 diabetes in remission) after sleeve gastrectomy.

The objectives of the current proof of concept, randomised, open label, crossover study is to investigate the effect of a fat-rich preload before a rich in carbohydrate meal after sleeve gastrectomy on

1) fasting, premeal, peak, nadir (lowest postprandial glucose levels) and postprandial glucose levels
2) fasting, premeal, peak and postprandial insulin levels
3) fasting, premeal, peak and postprandial c-peptide levels
4) fasting, premeal, peak and postprandial GLP-1 levels
5) symptoms suggestive of postprandial hypoglycaemia
6) symptoms suggestive of dumping syndrome
7) time to peak paracetamol levels after the MMTT as an index of gastric emptying

5. STUDY DESIGN

5.1 Trial Summary

This study is a proof-of-concept, randomised, open label, crossover study conducted over 29 days in male and female participants without diabetes (including those with type 2 diabetes in remission) who have undergone sleeve gastrectomy (SG).

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

- Group 1: will receive a fat rich pre-load and 80mls of water 30 minutes before the standardised MMTT with 1g dispersible paracetamol at visit 1, and then 100mls of water 30 minutes before the standardised MMTT with 1g dispersible paracetamol without a fat-rich preload at visit 2.
- Group 2: will receive 100mls of water 30 minutes before the standardised MMTT with 1g dispersible paracetamol without a fat-rich preload at visit 1, then a fat rich pre-load with 80mls of water 30 minutes before the standardised MMTT with 1g dispersible paracetamol at visit 2.

The participant flowchart is illustrated in Figure 1 (page 19).

Participants will attend a screening (familiarisation) visit (visit 0) prior to the start of the study followed by 2 visits over 8 (+7) days.

The screening (Familiarisation) visit (visit 0) will occur within approximately two weeks before Visit 1 (Baseline visit). Visit 0 (~2 hours) will comprise an eligibility assessment and a written informed consent obtained by an appropriately trained and delegated individual. In addition, blood samples will be obtained for HbA1c, full blood count (FBC), renal function and liver function. 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 and medication history will also be collected at this visit. A general physical examination will be performed by a trained delegated clinician.

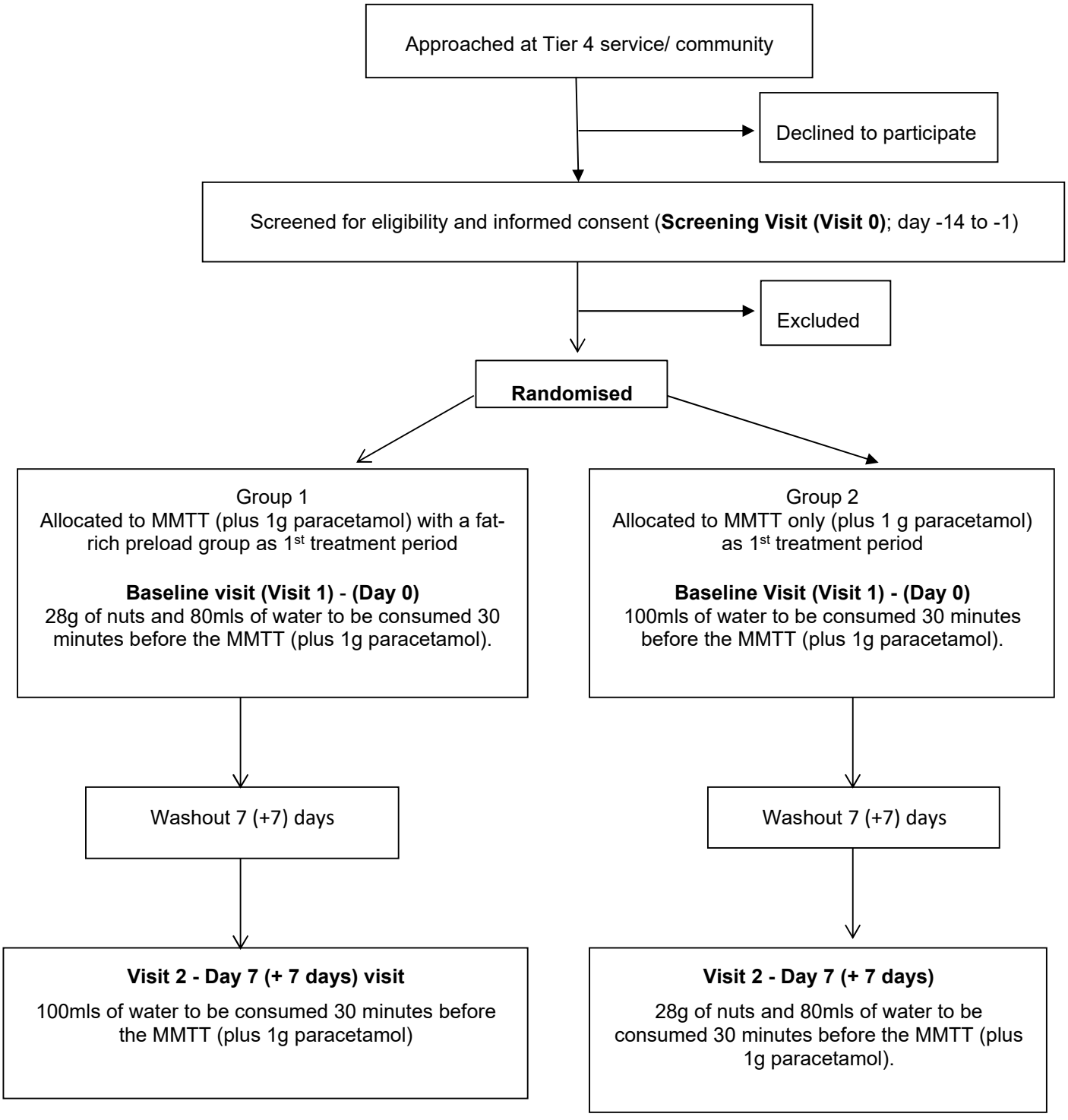
Visit 1 is the Baseline visit lasting approximately 4-5 hours and will take place at Leicester Diabetes Centre. Randomisation to one of the two treatment sequences will take place during this visit. Anthropometrics (weight including body fat %, height, blood pressure (BP), pulse rate) will be measured. 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 collection of multiple blood samples. Participants randomised to Group 1 will be asked to consume 28g of nuts

with 80mls of water over 10 minutes under supervision 30 minutes before initiation of the MMTT. Participants randomised to Group 2 will be asked to consume 100mls of water (similar volume to 28g nuts plus 80mls of water) over 10 minutes under supervision 30 minutes before initiation of the MMTT. Blood samples (for glucose, insulin, GLP-1 and paracetamol levels) will be collected via cannula: before the consumption of the fat-rich preload/water (-30'), before (-15') and immediately before (0') the MMTT and then 15', 30', 45', 60', 90', 120', 150' and 180' minutes after the MMTT consumption. Questionnaires on dumping symptoms and hypoglycaemia symptoms will be completed during the MMTT at the same time points that each blood sample is drawn; the questionnaires will be completed immediately after the blood draw.

Visit 2 (7 days after visit 1) will last approximately 4-5 hours and will be similar to visit 1. The only change is that participants who were allocated to Group 1 (standardised MMTT (plus 1g dispersible paracetamol) with a fat-rich preload) will now consume the standardised MMTT (plus 1g dispersible paracetamol) without the fat-rich preload sequence and vice versa will apply for those in Group 2. The rest of the procedures will be repeated as per Visit 1. Urine pregnancy test will be performed to all women of childbearing potential at Visit 2.

For the 24 hours before study visits 1 and 2, participants will be asked to refrain from completing any **moderate to vigorous** physical activity and consuming any alcohol. Participants will also be asked to refrain from consuming paracetamol 48 hours before study visits.

Figure 1. Participant flowchart



MMTT: Mixed meal tolerance test

5.2 Setting and timeframe

The study will be co-ordinated within the University Hospitals of Leicester NHS Trust and University of Leicester. Clinical measurement sessions will be co-ordinated by an 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 will take place by experienced research scientists at Leicester Diabetes Centre (LDC). Plasma glucose and serum paracetamol levels will be measured at the biochemistry 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 at community events. Recruitment will start in January 2022 and close in October 2023.

5.3 Primary and Secondary Endpoints

Primary endpoint

The difference in nadir (lowest) glucose levels after the standardised MMTT between the two treatment options (with or without fat-rich preload) after SG.

Secondary Endpoints

The secondary endpoints below will be measured at the time points defined in Table 1.

1. Difference in Area Under the Curve (AUC) $_{(-30\text{-}180)}$, fasting, premeal (defined as immediately before consumption of MMTT) and peak glucose levels after MMTT between the two treatment options (with or without fat-rich preload) after SG.
2. Difference in AUC$_{(-30\text{-}180)}$ insulin, fasting, premeal and peak insulin levels after MMTT between the two treatment options (with or without fat-rich preload) after SG.
3. Difference in AUC$_{(-30\text{-}180)}$ GLP-1, fasting, premeal and peak GLP-1 levels after MMTT between the two treatment options (with or without fat-rich preload) after SG
4. Difference in AUC$_{(-30\text{-}180)}$ c-peptide, fasting, premeal and peak c-peptide levels after MMTT between the two treatment options (with or without fat-rich preload) after SG
5. Difference in the ratio AUC$_{(-30\text{-}180)}$ insulin/AUC$_{(-30\text{-}180)}$ glucose between the two treatment options (with or without fat-rich preload) after SG
6. Difference in the ratio AUC$_{(-30\text{-}0)}$ insulin/AUC$_{(-30\text{-}0)}$ glucose between the two treatment options (with or without fat-rich preload) after SG

7. Difference in the ratio $AUC_{(0-30)}$ insulin/$AUC_{(0-30)}$ glucose between the two treatment options (with or without fat-rich preload) after SG
8. Difference in the ratio $AUC_{(60-180)}$ insulin/$AUC_{(60-180)}$ glucose between the two treatment options (with or without fat-rich preload) after SG
9. Difference in the ratio $AUC_{(-30-180)}$ c-peptide/$AUC_{(-30-180)}$ glucose after MMTT between the two treatment options (with or without fat-rich preload) after SG
10. Difference in the ratio $AUC_{(-30-0)}$ c-peptide/$AUC_{(-30-0)}$ glucose after MMTT between the two treatment options (with or without fat-rich preload) after SG
11. Difference in the ratio $AUC_{(0-30)}$ c-peptide/$AUC_{(0-30)}$ glucose after MMTT between the two treatment options (with or without fat-rich preload) after SG
12. Difference in the ratio $AUC_{(60-180)}$ c-peptide/$AUC_{(60-180)}$ glucose after MMTT between the two treatment options (with or without fat-rich preload) after SG
13. Difference in the ratio of maximum/minimum plasma glucose after MMTT between the two treatment options (with or without fat-rich preload) after SG
14. Difference in $AUC_{(-30-180)}$ of Sigstad score, fasting, premeal and peak Sigstad score after MMTT between the two treatment options (with or without fat-rich preload) after SG
15. Difference between $AUC_{(-30-180)}$ of Edinburgh Hypoglycaemia Scale score, fasting, premeal and peak Edinburgh Hypoglycaemia Scale score after MMTT between the two treatment options (with or without fat-rich preload) after SG
16. Difference in $AUC_{(0-180)}$ paracetamol, and peak paracetamol levels after MMTT between the two treatment options (with or without fat-rich preload) after SG
17. Difference at the time to peak paracetamol levels after MMTT between the two treatment options (with or without fat-rich preload) after SG
18. The Amount of glucose (in grams) needed to restore euglycaemia between the two treatment options (with or without fat-rich preload) after SG
19. The number of MMTTs needed to be terminated early because of hypoglycaemia (blood glucose or capillary glucose levels ≤3.0mmol/l) with or without fat-rich preload after SG.

6. TRIAL PARTICIPANTS

6.1 Overall Description of Trial Participants:

Male and female subjects aged ≥ 18 years, without diabetes (including those with type 2 diabetes in remission) after SG and at least one year postoperatively (n=12) 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

1. Aged ≥18 years old
2. Subjects ≥1 year after sleeve gastrectomy (SG)
3. Able to understand written and spoken English
4. Able to give informed consent
5. Happy for their GP to be notified of their study participation

6.3 Exclusion criteria:

1. Use of any glucose-lowering medication (including insulin)
2. Adrenal insufficiency and/or substitution with glucocorticoids
3. eGFR ≤ 45 ml/min/1.73m^2
4. weight ≤ 50kg
5. Recent active infection (an active infection will be any infection over the last 10 days)
6. 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
7. People with allergy or severe intolerance to the mixed meal tolerance test as assessed by the clinician (e.g., severe milk protein allergy, lactose and gluten intolerance)
8. People with allergy or intolerance to paracetamol or to nuts
9. Other bariatric procedure except of SG
10. Previous major revisional bariatric surgery (except of previous gastric band which has been removed)
11. Hb <100 g/L at screening blood tests
12. HbA1C ≥6.5% or ≥48 mmol/L at screening blood tests
13. Currently pregnant or breastfeeding
14. Diagnosis of Type 1 Diabetes

15. Current diagnosis of Type 2 Diabetes (defined as HbA1C ≥6.5%/ ≥48 mmol/L at screening blood tests or HbA1C <6.5%/ <48 mmol/l at screening blood tests but on glucose lowering medications over last 3 months)
16. Patients with diagnosis of Epilepsy
17. Participating in another Clinical Trials of Investigational Medicinal Products (CTIMP) study within <1 month of screening
18. Having a formal previous diagnosis of postprandial hypoglycaemia
19. Currently on Metoclopramide, domperidone or colestyramine as they can affect paracetamol absorption as per SPC (Summary of Product Characteristics) for paracetamol.
20. Currently on acarbose, diazoxide, octreotide or other treatment for postprandial hypoglycaemia
21. Any concurrent condition, in the judgment of investigator and/ or GP practitioner, 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 (PIS) 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. Study staff may also identify potential participants through the Leicester Diabetes Centre volunteer's database if they have consented to be contacted to take part in future ethically approved research. 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 informed study consent.

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 East Midlands Clinical Research Network by setting up PIC sites from Primary care and Secondary care within the East & West Midlands area.

A team of five consultants in Upper Gastrointestinal (UGI) surgery provide bariatric surgery to the local population (Leicester/Leicestershire/Rutland) in University Hospitals of Leicester NHS trust, and in the last 10 years, more than 300 patients have been operated with SG. This study will recruit mainly from secondary care bariatric surgery clinics (Tier 4 follow-up clinics) at UHL NHS trust, University Hospitals of Derby and Burton NHS Foundation Trust, University Hospitals of Coventry & Warwickshire and University Hospitals of Birmingham, as well as from private practice clinics (surgical clinics) and primary care (GP Practices) for subjects who have been discharged from Tier 4 services.

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 and a follow-up call to participants approximately 2 weeks after each mailing. If GP practices are willing to

add notes and/or reminders to patient records within SystmOne 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.

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.

The following recruitment activities will be used:

1. Primary care
    a. GP practices (within the East & West Midlands area)
    Local CRN will support recruitment from Primary care; Study will be advertised online by CRN whereby GP's can submit Expressions of Interest. Expressions of Interest will be sent through to the study team and study packs will be sent out to GP practices to pass onto potential participants. Packs will contain study Patient Invitation Letter, Patient Information Sheet, and a reply slip. If the study team are required to visit a practice for a GP visit, they must adhere to Government (COVID-19) guidelines and any additional measurements in place within GP practices.
2. Secondary care
    a. Attendance at UHL Outpatients Clinics, for example:
        i. Bariatric surgery follow-up clinics
        ii. Dietetic clinics
        iii. Obstructive sleep apnoea
        iv. Chemical pathology clinics
    b. Secondary Care Databases – A 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- Database of discharged patients who have been seen in private hospitals (e.g. Spire) but have been discharged from their care. The consultant will inform these discharged patients of the study by mailing them with relevant study material. Any agreements required between the lead research site (UHL) and the consultant as an independent party will be fully executed before the consultant makes contact with these discharged patients.

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 SG if they have consented to being contacted regarding future ethically approved research.

4. Key people in community/community events and meetings

   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. Due to the current COVID-19 situation, study team may not be able to participate or hold Face to Face meetings. Alternatively, the study team can circulate the study poster and other supporting documents to key people within the community. Those interested in learning more about the study will be able to contact the study team directly.

5. Recruitment/health fairs in community

   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. The study team will only participate in community events if possible and will ensure they follow COVID-19 guidelines and any other measures in place to ensure safety of themselves and others. Alternatively, the study team can contact people within the community to advertise Study posters on community websites to minimise Face to Face contact.

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 participant flow through the study is highlighted in Figure 1 (page 19).

Visit 0 (Screening Visit, -14 days to –1 day), Visit 1 (Baseline Visit - 0 days), Visit 2 [(7+(7) days).

7.4 Informed Consent (Screening Visit, Visit 0)

Written informed consent will be obtained from all participants prior to undertaking any study procedures and only after they have had sufficient time (at least 24 hours) to read through the patient information Sheet (PIS) and ask any questions. A member of the study team 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. One copy of the 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. 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 a member of the study team.

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.

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 a standardised MMTT between the two treatment options (with or without fat-rich preload).

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

Before study visits 1 and 2, participants will be required to fast for 10 hours (from ~23:00 the previous day). Moreover, before study visits 1 and 2, there will be a 24 hour standardisation period. There will also be a 48 hour standardisation period before study visit 1 and 2.

The standardisation plans are described below:

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 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.

Participants will also be asked to avoid taking Paracetamol tablets for at least 48-h before the study for both visit 1 and 2

Fat-rich preload standardisation

Thirty minutes before the standardised mixed meal test (described below), participants will consume (based on the randomisation sequence) either 28g of nuts with 80mls of water (those randomised to receive a fat-rich preload; Group 1) or 100mls of water (those randomised not to receive a fat-rich preload; Group 2). The fat-rich preload or water will be consumed over 10 minutes and under supervision.

Mixed meal test standardisation (plus 1g dispersible paracetamol)

Participants will be provided with a standardised mixed meal test which will comprise 220mls of Nutricia Fortisip Milkshake (330 kcal, 12.76g fat, 40.5g carbohydrates, 13.2g protein). One gram of paracetamol will be dispersed in 20mls of water and will be added into the standardised MMTT at both visits in order to assess the gastric emptying through a paracetamol absorption test, a validated method of assessing gastric emptying after SG.

Participants will be asked to consume the standardised MMTT (plus 1g of dispersible paracetamol) over 10 minutes and under supervision.

Glucose levels will be measured at the University Hospitals of Leicester NHS Trust laboratories and then samples disposed of in accordance with the Human Tissue Authority's Code of Practice.

Blood glucose levels will be measured at the following time points: -30' (immediately before consumption of the fat-rich preload/ water), -15', 0' (immediately before consumption of the MMTT) and then starting from the 'time of the last mouthful of the MMTT' at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes. A total of 11 samples will be taken during each visit. A total of 138 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 samples for insulin, Glucagon Like Peptide-1 and paracetamol levels will be measured at the following time points: immediately before initiation of consumption of the fat-rich preload/water (-30'), before (-15') and immediately before (0') the MMTT and 15', 30', 45', 60', 90', 120', 150' and 180' after the MMTT consumption (to correspond with the glucose measurements).

Blood samples for insulin 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 tubes (EDTA 4.9ml, 1 tube at each time point) containing 250 microlitres aprotinin to ensure sample viability for hormone/metabolite biochemical analysis. Blood collection tubes will be kept on ice prior to their use. Samples will be transferred to Research Scientists based at the Leicester Diabetes Centre laboratories, University Hospitals of Leicester for measurement of insulin levels.

Blood samples for paracetamol levels will be collected into syringes and blood will be added to serum tubes immediately after collection. Samples will be transferred to the University Hospitals of Leicester laboratories for measurement of serum paracetamol levels.

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 using dry ice to Loughborough University for subsequent analysis.

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 compliance with the Human Tissue Act and to ensure that they will be used following the conditions that participants have given consent for.

Study Questionnaires

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

Edinburgh Hypoglycaemia Scale (EHS) (35) – This will assess symptoms of hypoglycaemia during the mixed meal tolerance test at visits 1 and 2; time points -30' (before consumption of the fat-rich preload/water), -15', 0' (immediately before consumption of MMTT), 15' (after consumption of the last mouthful of the MMTT), 30', 45', 60', 90', 120', 150', 180'. This will be completed at the time-points above once blood samples have been drawn.

Sigstad dumping score (7,36) – This will assess symptoms of dumping during the mixed meal tolerance test at visits 1 and 2: time points -30' (before consumption of the fat-rich preload/ water), -15', 0' (immediately before consumption of MMTT), 15' (after consumption of the last mouthful of the MMTT), 30', 45', 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). Changes to medical history will be recorded at subsequent visits.

Physical Examination (Visit 0)

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

Concomitant medication (visit 0)

Use of any medication will be collected and changes to medications will be recorded at subsequent visits.

7.6 Randomisation (Visit 1)

Randomisation will take place at the level of the individual using an independent online computerised randomisation service (sealedenvelope.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) a fat-rich preload with 1g dispersible paracetamol 30 minutes before the MMTT at visit 1, followed by MMTT with 1g dispersible paracetamol without a fat-rich preload after a "washout" period of 7 days

(Group 2) MMTT with 1g dispersible paracetamol without a fat-rich preload followed by a MMTT with 1g dispersible paracetamol with a fat-rich preload 30 minutes before the MMTT after a "washout" period of 7 days.

Participants will be informed of their randomisation assignment during the Baseline Visit. A letter from the baseline visit will also be sent to the participant's GP notifying them of their patient's participation in the study which will also inform them of the results.

7.7 Subsequent Assessments

Table 1 (pages 34-35) 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. Written informed consent will be obtained by appropriately and delegated individual. 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),

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 on the previous night) for 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 Adverse Events (AE) /Serious Adverse Events (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 a fat-rich preload 30 minutes before the MMTT based on treatment sequence will take place. Blood sampling for measurement of (plasma glucose, insulin, GLP-1 and serum paracetamol levels) will be collected via cannula: before the consumption of the fat-rich preload/water (-30'), before (-15') and immediately before (0') before the MMTT plus 1g paracetamol and then 15' 30', 45', 60', 90', 120', 150' and 180' minutes after the MMTT consumption . Blood glucose levels will also be measured through a glucose meter at the same time points. An extra capillary blood glucose measurement 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 Capillary Blood Glucose (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 the patient is clinically well, blood sampling will be performed for plasma glucose and the rest of the biochemical markers immediately before treatment of hypoglycaemia]. During the mixed meal test, patients will complete validated questionnaires at the same time points that each blood sample is drawn, on dumping symptoms (7,36), as well as on hypoglycaemia symptoms (35); the questionnaires will be completed immediately after the blood draw. Severe neuroglycopenic symptoms (defined as ≥5 on the Edinburgh Hypoglycaemia Scale for 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 on the previous night) for 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 and blood samples will be collected at the same time points as Visit 1. A 3-h MMTT (plus 1g paracetamol) with or without a fat-rich preload based on treatment sequence (opposite to what participants received at the visit 1), will take place.

An extra capillary blood glucose 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 the MMTT 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 the patient is clinically well, blood sampling will be performed for plasma glucose and the rest of biochemical markers immediately before treatment of hypoglycaemia]. During the mixed meal test, patients will complete validated questionnaires at the same time points that each blood sample is drawn, on dumping symptoms, as well as on hypoglycaemia symptoms; the questionnaires will be completed immediately after the blood draw. Severe neuroglycopenic symptoms (defined as ≥5 on the Edinburgh Hypoglycaemia Scale for 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.

7.8 Definition of End of Trial

The end of trial is the date when analysis of study data and samples 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
- Pregnancy

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 the 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, 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
	-14 days to -1 day	0 days	7 (+7) days
Study procedures			
Inclusion/exclusion criteria	X	X#	X#
Informed consent	X		
Randomisation		X	
Clinician/ Nurse appointment	X	X	X
Cannula insertion		X	X
3-h mixed meal tolerance test with 1g paracetamol (with or without fat-rich preload, as per randomization)		X	X
Blood sampling	X(1)	X(11)	X(11)
Blood glucose monitoring		X(11)	X(11)
Filling CRF forms	X	X	X
Centrifuge of samples		X(11)	X(11)
Storing of samples		X(11)	X(11)
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

	-14 days to -1 day	0 days	7 (+7) 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(11)	X(11)
Dumping questionnaire		X(11)	X(11)
Physical Activity and alcohol question and fasting (last 24 hours)		X	X
Paracetamol question (48 hours)		X	X
AE/SAE recording (including hypoglycaemia)		X	X

X#: Check for any changes in patient's exclusion criteria [weight 50kg, changes in medications (use of oral or injectable steroids, use of glucose lowering medications, use of medications described at exclusion criteria affecting paracetamol absorption, use of medications for treatment of postprandial hypoglycaemia), changes in medical history in accordance with exclusion criteria (for example new diagnosis of diabetes, epilepsy or adrenal insufficiency, pregnancy/breastfeeding).

Patients who experienced episode of postprandial hypoglycaemia during the MMTT (symptomatic or asymptomatic) and during the study, will be able to continue and complete the study.X*: For all female patients of childbearing potential.

8. TREATMENT OF STUDY PARTICIPANTS

8.1 Description of Study Treatment

The study treatment is 28g of nuts. This amount of nuts is equivalent to a serving. Moreover, paracetamol 1g dispersed in 20mls of water will be added to the MMTT. The paracetamol dosage will not exceed the stated recommendations of the British National Formulary.

All potential contra-indications to use of nuts (such as allergy to nuts) and severe allergies or intolerances to the MMTT (Nutricia Fortisip Milkshake). Paracetamol use will be checked as part of the eligibility criteria.

8.2 Storage and dispense of Study Treatment

28g nuts (prepacked) will be stored at room temperature at Leicester Diabetes Centre. The MMTT (Nutricia Fortisip Milkshake will be stored in the study fridge within the Leicester Diabetes Centre.

Dispersible paracetamol will be stored in a designated, restricted area for medication storage at Leicester Diabetes Centre, Leicester General Hospital. Dispersible paracetamol 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, 1g of paracetamol will be dispersed in 20mls of water and will be added into the MMTT which will be consumed by the participant.

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 corticosteroids, 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 the intervention provided during the study.

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 intervention, whether or not considered related to the study intervention.

Adverse Reaction (AR)

All untoward and unintended responses to the study intervention. The phrase "responses to the study intervention" means that a causal relationship between the study intervention (nuts consumption) 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 intervention 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.

Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information (SPC) will be reported as a SUSAR.

The study is using Paracetamol at a dose in accordance with the recommendations of BNF and the SPC, therefore no SUSARs are expected.

Expected Serious Adverse Events/Reactions (SAE)

No SAEs are expected during this study from the use of a fat-rich snack (nuts) , the consumption of Nutricia Fortisip, and the use of paracetamol in accordance with the British National Formulary (BNF) and the SPC. However, all SAEs will be reported to the Sponsor (except of planned routine and elective surgery).

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 the patient is clinically well, blood sampling will be attempted for plasma glucose and the rest of the biochemical markers immediately before treatment of hypoglycaemia]. If the participant is asymptomatic or having autonomic/other symptoms (in accordance with Appendix 3) he/she 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 PHH after SG.

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 fat-rich snack (nuts) and medication (paracetamol) 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 OR paracetamol will be assessed by a medically qualified investigator.

9.4 Reporting Procedures for Serious Adverse Events

All SAEs must be reported to the Sponsor immediately and within 24 hours of becoming aware of the event. 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 (plus 1 g of paracetamol) with and without a fat-rich preload 30 minutes before the MMTT 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. If any safety event occurs it shall be reported according to the process described above. Hypoglycaemia during the MMTT will also be treated as described above. If patients experience autonomic symptoms suggestive of early dumping syndrome (during the first 15-30 minutes after ingestion of the MMTT usually), they will be asked to lie comfortably until symptoms improve (the usual duration of symptoms is 15 minutes).

In the case of clinically relevant readings being collected (e.g. blood pressure ≥170/95 mm Hg 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 mm Hg at any visit or K+ >7.0 mmol/l during screening visit) will be referred by a doctor to the appropriate services depending on the nature and history of the condition.

9.6 Response to the COVID-19 global pandemic

During the COVID-19 Global pandemic study based risk assessments will be conducted and measures will be put into place ensure safety of participants and staff. Study based risk assessments will be completed and in agreement with sponsor, study team and NHS Host Organisation will also be put in place to ensure appropriate measures are in place to carry out study measurements in a safe manner.

All study visits will take place as face to face due to the nature of measurements and procedures required for this study. However to ensure safety of participants and staff, staff will be in PPE and participants will need ensure they have face coverings on before entering the Leicester Diabetes Centre.

All clinical areas used for research visits will also have risk assessments completed to ensure mitigations are in place where risks are identified. Participants will also undergo a COVID-19 check which includes a temperature check and a COVID-19 questionnaire put in place to ensure safety of staff and participants. This will be carried out prior to starting any study visits.

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 after the standardised MMTT with or without a fat-rich preload after SG for two period crossover design. Data will be collected at time points (minutes) -30' (immediately before nut consumption), -15', 0' (immediately before MMTT meal consumption), 15', 30', 45', 60', 90', 120', 150', 180' after MMTT consumption. The AUC [(Area Under the Curve) calculated using trapezium rule] will be used for summarising the response to the additional fat-rich preload before 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. 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 and the time periods covered are relatively short, 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 purpose of this study is to test the concept of the effectiveness of a fat-rich preload before a carbohydrate-rich meal on glucose homeostasis after SG in humans. It is not a definitive test of the effectiveness of potential treatment; therefore a power calculation is not computed. The number of subjects within each treatment group after SG (n=12) was chosen to provide reasonable information on the key objectives of the study based on previous pilot studies (23-25, 37,38). Low dropout rate is expected due to short duration of the study.

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 a subject, The Study will also be subjected to an additional COVID-19 Sponsor Risk Assessment prior to opening to make sure adequate measures are in place to ensure safety of Participants and staff are in place to deliver the study safely.

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 available for monitoring, audit and inspections by the Ethics Committee, the sponsor and 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 hard and electronic copies research documentation, data, and blood samples collected 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 servers 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

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

Operational meetings will include the chief investigator, other senior investigators and the day-to-day project management team. The group will meet regularly 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 and/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 authorising REC will be acknowledged.

17. REFERENCES

1) Haslam DW, James WP. Obesity. Lancet. 2005 Oct 1; 366(9492):1197-209.
2) Ahmad A, Laverty AA, Aasheim E, Majeed A, Millett C, Saxena S. Eligibility for bariatric surgery among adults in England: analysis of a national cross-sectional survey. JRSM Open. 2014 Jan 7;5(1).
3) http://www.noo.org.uk/NOO_about_obesity/severe_obesity
4) Sjöström L. Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery. J Intern Med. 2013 Mar;273(3):219-34.
5) IFSO Worldwide Survey 2016: Primary, Endoluminal, and Revisional Procedures. Angrisani L, Santonicola A, Iovino P, Vitiello A, Higa K, Himpens J, Buchwald H, Scopinaro N. Obes Surg. 2018 Dec;28(12):3783-3794.
6) Ben-Porat T, Elazary R, Goldenshluger A, Sherf Dagan S, Mintz Y, Weiss R. Nutritional deficiencies four years after laparoscopic sleeve gastrectomy-are supplements required for a lifetime? Surg Obes Relat Dis. 2017 Jul;13(7):1138-1144
7) Tzovaras G, Papamargaritis D, Sioka E, Zachari E, Baloyiannis I, Zacharoulis D, Koukoulis G. Symptoms suggestive of dumping syndrome after provocation in patients after laparoscopic sleeve gastrectomy. Obes Surg. 2012 Jan;22(1):23-8.
8) Sun W, Zhang Y, Shen Q, Zhang W, Yao Q, Yang Y. Prevalence and risk factors for symptoms suggestive of hypoglycemia and early dumping syndrome after sleeve gastrectomy. Surg Obes Relat Dis. 2019 Sep;15(9):1439-1446
9) Papamargaritis D, Koukoulis G, Sioka E, Zachari E, Bargiota A, Zacharoulis D, Tzovaras G. Dumping symptoms and incidence of hypoglycaemia after provocation test at 6 and 12 months after laparoscopic sleeve gastrectomy. Obes Surg. 2012 Oct;22(10):1600-6
10) Capristo E, Panunzi S, De Gaetano A, Spuntarelli V, Bellantone R, Giustacchini P, Birkenfeld AL, Amiel S, Bornstein SR, Raffaelli M, Mingrone G. Incidence of Hypoglycemia After Gastric Bypass vs Sleeve Gastrectomy: A Randomized Trial. J Clin Endocrinol Metab. 2018 Jun 1;103(6):2136-2146
11) Belligoli A, Sanna M, Serra R, Fabris R, Pra' CD, Conci S, Fioretto P, Prevedello L, Foletto M, Vettor R, Busetto L. Incidence and Predictors of Hypoglycemia 1 Year After Laparoscopic Sleeve Gastrectomy. Obes Surg. 2017 Dec;27(12):3179-3186.
12) Lee CJ, Clark JM, Schweitzer M, Magnuson T, Steele K, Koerner O, Brown TT. Prevalence of and risk factors for hypoglycemic symptoms after gastric bypass and sleeve gastrectomy. Obesity (Silver Spring). 2015 May;23(5):1079-84
13) Yaqub A, Smith EP, Salehi M. Hyperinsulinemic hypoglycemia after gastric bypass surgery: what's up and what's down? Int J Obes (Lond). 2017 Oct 13.
14) Emous M, Ubels FL, van Beek AP. Diagnostic tools for post-gastric bypass hypoglycaemia. Obes Rev. 2015 Oct;16(10):843-56.
15) Emous M, Wolffenbuttel BHR, Totté E, van Beek AP. The short- to mid-term symptom prevalence of dumping syndrome after primary gastric-bypass surgery and its impact on health-related quality of life. Surg Obes Relat Dis. 2017 Sep;13(9):1489-1500
16) Varma S, Clark JM, Schweitzer M, Magnuson T, Brown TT, Lee CJ. Weight regain in patients with symptoms of post-bariatric surgery hypoglycemia. Surg Obes Relat Dis. 2017 Oct;13(10):1728-1734
17) Marsk R, Jonas E, Rasmussen F, Näslund E. Nationwide cohort study of post-gastric bypass hypoglycaemia including 5,040 patients undergoing surgery for obesity in 1986-2006 in Sweden. Diabetologia. 2010 Nov;53(11):2307-11
18) Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, Lamonte MJ, Stroup AM, Hunt SC. Long-term mortality after gastric bypass surgery. N Engl J Med. 2007 Aug 23;357(8):753-61

19) Nannipieri M, Belligoli A, Guarino D, Busetto L, Moriconi D, Fabris R, Mari A, Baldi S, Anselmino M, Foletto M, Vettor R, Ferrannini E. Risk Factors for Spontaneously Self-Reported Postprandial Hypoglycemia After Bariatric Surgery. J Clin Endocrinol Metab. 2016 Oct;101(10):3600-3607.
20) Rebelos E, Moriconi D, Scalese M, Denoth F, Molinaro S, Siciliano V, Anselmino M, Taddei S, Ferrannini E, Nannipieri M. Impact of Postprandial Hypoglycemia on Weight Loss After Bariatric Surgery. Obes Surg. 2020 Mar 4. [Epub Ahead of Print]
21) Papamargaritis D, Zacharoulis D, Sioka E, Zachari E, Bargiota A, Koukoulis G, Tzovaras G. Differences in Anthropometric and Metabolic Parameters Between Subjects with Hypoglycaemia and Subjects with Euglycaemia After an Oral Glucose Tolerance Test Six Months After Laparoscopic Sleeve Gastrectomy. Obes Surg. 2016 Nov;26(11):2747-2755.
22) Øhrstrøm CC, Worm D, Hansen DL. Postprandial hyperinsulinemic hypoglycemia after Roux-en-Y gastric bypass: an update. Surg Obes Relat Dis. 2017 Feb;13(2):345-351.
23) Øhrstrøm CC, Worm D, Højager A, Andersen D, Holst JJ, Kielgast UL, Hansen DL. Postprandial hypoglycaemia after Roux-en-Y gastric bypass and the effects of acarbose, sitagliptin, verapamil, liraglutide and pasireotide. Diabetes Obes Metab. 2019 May 30.[Epub Ahead of Print]
24) Valderas JP, Ahuad J, Rubio L, Escalona M, Pollak F, Maiz A. Acarbose improves hypoglycaemia following gastric bypass surgery without increasing glucagon-like peptide 1 levels. Obes Surg. 2012 Apr;22(4):582-6.
25) Ritz P, Vaurs C, Bertrand M, Anduze Y, Guillaume E, Hanaire H. Usefulness of acarbose and dietary modifications to limit glycemic variability following Roux-en-Y gastric bypass as assessed by continuous glucose monitoring. Diabetes Technol Ther. 2012 Aug;14(8):736-40.
26) Melissas J, Daskalakis M, Koukouraki S, Askoxylakis I, Metaxari M, Dimitriadis E, Stathaki M, Papadakis JA. Sleeve gastrectomy-a "food limiting" operation. Obes Surg. 2008 Oct;18(10):1251-6
27) Sioka E, Tzovaras G, Perivoliotis K, Bakalis V, Zachari E, Magouliotis D, Tassiopoulou V, Potamianos S, Kapsoritakis A, Poultsidi A, Tepetes K, Chatzitheofilou C, Zacharoulis D. Impact of Laparoscopic Sleeve Gastrectomy on Gastrointestinal Motility. Gastroenterol Res Pract. 2018 Apr 5;2018
28) Svane MS, Bojsen-Møller KN, Martinussen C, Dirksen C, Madsen JL, Reitelseder S, Holm L, Rehfeld JF, Kristiansen VB, van Hall G, Holst JJ, Madsbad S. Postprandial Nutrient Handling and Gastrointestinal Hormone Secretion After Roux-en-Y Gastric Bypass vs Sleeve Gastrectomy. Gastroenterology. 2019 May;156(6):1627-1641.
29) Papamargaritis D, le Roux CW, Sioka E, Koukoulis G, Tzovaras G, Zacharoulis D. Changes in gut hormone profile and glucose homeostasis after laparoscopic sleeve gastrectomy. Surg Obes Relat Dis. 2013 Mar-Apr;9(2):192-201.
30) Peterli R, Steinert RE, Woelnerhanssen B, Peters T, Christoffel-Courtin C, Gass M, Kern B, von Fluee M, Beglinger C. Metabolic and hormonal changes after laparoscopic Roux-en-Y gastric bypass and sleeve gastrectomy: a randomized, prospective trial. Obes Surg. 2012 May;22(5):740-8.
31) Nesti L, Mengozzi A, Tricò D. Impact of Nutrient Type and Sequence on Glucose Tolerance: Physiological Insights and Therapeutic Implications. Front Endocrinol (Lausanne). 2019 Mar 8;10:144.
32) Gentilcore D, Chaikomin R, Jones KL, Russo A, Feinle-Bisset C, Wishart JM, et al. Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. J Clin Endocrinol Metab. 2006;91:2062–7.
33) Cunningham KM, Read NW. The effect of incorporating fat into different components of a meal on gastric emptying and postprandial blood glucose and insulin responses. Br J Nutr. 1989; 61:285–90.
34) Crouch MA, Slater RT. Almond “Appetizer” effect on glucose tolerance test (GTT) results. J Am Board Fam Med. 2016; 29:759–66.

35) Abrahamsson N, Börjesson JL, Sundbom M, Wiklund U, Karlsson FA, Eriksson JW. Gastric Bypass Reduces Symptoms and Hormonal Responses in Hypoglycemia. Diabetes. 2016 Sep;65(9):2667-75
36) Laurenius A, Werling M, Le Roux CW, Fändriks L, Olbers T. More symptoms but similar blood glucose curve after oral carbohydrate provocation in patients with a history of hypoglycemia-like symptoms compared to asymptomatic patients after Roux-en-Y gastric bypass. Surg Obes Relat Dis. 2014 Nov-Dec;10(6):1047-54.
37) Tack J, Aberle J, Arts J, Laville M, Oppert JM, Bender G, Bhoyrul S, McLaughlin T, Yoshikawa T, Vella A, Zhou J, Passos VQ, O'Connell P, Van Beek AP. Safety and efficacy of pasireotide in dumping syndrome-results from a phase 2, multicentre study. Aliment Pharmacol Ther. 2018 Jun;47(12):1661-1672
38) Craig CM, Liu LF, Nguyen T, Price C, Bingham J, McLaughlin TL. Efficacy and pharmacokinetics of subcutaneous exendin (9-39) in patients with post-bariatric hypoglycaemia. Diabetes Obes Metab. 2018 Feb;20(2):352-361.

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

“Since 11pm last night have you eaten or drank anything apart from water?”- Yes/No

“In the Last 48 hours have you taken any Paracetamol?”- 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 behaviour							
Speech Difficulty							
Incoordination							
Malaise symptoms							
Nausea							
Headache							