# The effect of acute aerobic exercise on the time spent in hypoglycaemia after bariatric surgery (The BariEX Study)

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## Confidentiality Statement

All information contained within this protocol is regarded as, and must be kept confidential. No part of it may be disclosed by any Receiving Party to any Third Party, at any time, or in any form without the express written permission from the Chief Author/Investigator and/ or Sponsor.

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## AMENDMENT HISTORY

| Amendment No. | Protocol Version No. | Date issued | Author(s) of changes | Details of Changes made |
|---|---|---|---|---|
| | | | | |
| SA01 | 2.0 | 07.12.2021 | Dr. Louisa Herring<br>Dr. Dimitris Papamargaritis<br>Dr. Nicole Coull | • Addition to the exclusion criteria to exclude participants with severe intolerance to standardised meals and Mixed Meal Tolerance Tests (MMTT), to add clarification around use of steroids and to remove the 'over the last 6 months' statement for eGFR.<br>• Removal of indirect calorimetry measurement from visit 0 to use Mifflin equation instead.<br>• Addition of Research Registries to the recruitment strategy.<br>• Correction of wording around staff members performing maximal exercise tests.<br>• Removal of 3-day diet diary in the washout period.<br>• Minor corrections of typos and missing text.<br>• Shortening/reformatting of the short and full participant information |

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| | | | | sheets to make these more appealing to potential participants and removal of reference to indirect calorimetry.<br>• Minor change to the wording contained within the baseline appointment letter.<br>• Change to study start and end date due to COVID-19. |
| NSA02 | 2.1 | 22.03.2022 | Dr. Louisa Herring<br>Dr. Dimitris Papamargaritis | • Treatment day breakfast meal changed from 30 to 15% of participant's estimated daily energy requirements.<br>• Participants will be given extra 5 minutes for mixed meal tolerance test consumption if needed. |
| NSA03 | 2.2 | 28.04.2022 | Dr. Louisa Herring<br>Dr. Dimitris Papamargaritis | • Exclusion criteria changes: history of type 2 diabetes diagnosis changed to current type 2 diabetes diagnosis.<br>• Information about glucose samples analysis updated. |
| NSA04<br>&<br>NA05 | 2.3 | 01.12.2022 | Dr. Louisa Herring<br>Dr. Dimitris Papamargaritis | • Update to recruitment strategy to extend use of PIC sites (primary and secondary care) into the West Midlands, University Hospitals of Birmingham and Luton & Dunstable University Hospital (Bedfordshire Hospitals NHS Trust). Update to appointment |

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| | | | | letters to reflect updated travel re-imbursement costs.<br>• Minor corrections of typos. |
| NSA06 | 2.4 | 24.10.2023 | Dr Louisa Herring<br>Dr Dimitris Papamargaritis | • The study title has been updated to "The effect of acute aerobic exercise on the time spent in hypoglycaemia after bariatric surgery (The BariEX Study).<br>• Update to the inclusion criteria to include patients who have also had a Sleeve Gastrectomy (SG) bariatric procedure.<br>• All relevant study documents have been updated to reflect the inclusion of Sleeve Gastrectomy (SG) bariatric procedure.<br>• Extension to the recruitment end date to 31.07.2024 and the overall study end date to 31.10.2024 to allow time to recruit to time and target. |

## 1. SYNOPSIS

| | |
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| **Study Title** | The effect of acute aerobic exercise on the time spent in hypoglycaemia after bariatric surgery (The BariEX Study) |
| **Internal ref. no.** | EDGE 135117 |
| **Trial Design** | Randomised crossover study |
| **Trial Participants** | Individuals ≥12 months after bariatric surgery (Roux-en-Y gastric bypass or sleeve gastrectomy), aged ≥18 to <75 years without diabetes |
| **Planned Sample Size** | 18 participants |
| **Study duration** | 24 months |
| **Planned Recruitment Period** | 24 months |
| **Primary Objective** | To investigate the effect of a single bout of aerobic exercise (AEX) on time spent in hypoglycaemia (HYPO) (defined as glucose levels <3.0mmol/l) and on glucose homeostasis during the first 24 hours(h) after an AEX session |
| **Secondary Objectives** | 1. To assess the difference in continuous glucose monitoring (CGM) metrics for clinical care between the AEX and control intervention (prolonged sitting) for the 24-h period following completion of the intervention (for example, CGM mean glucose concentrations, glucose variability, time spent in hyperglycaemia and the time in range).<br>2. To assess the differences in nadir interstitial glucose levels, in risk for HYPO (assessed through the low blood glucose index), in risk of hyperglycaemia (assessed through the high blood glucose index), in the number of episodes of HYPO (glucose levels <3.0mmol/l) and in the number of episodes requiring HYPO treatment between the two interventions for the 24h period following completion of each intervention.<br>3. To assess the change in the peak, nadir and postprandial [estimated through area under the curve (AUC)] blood glucose levels as well as the change in peak and postprandial (estimated through AUC) insulin levels after the standardised lunch between the two conditions. |
| **Primary Endpoint** | Assessment of the time spent in hypoglycaemia (defined as glucose levels <3.0mmol/l) during the first 24h after completion of AEX or control condition. |
| **Secondary Endpoints** | Analysis between AEX and control interventions for the following;<br>1. CGM metrics (including mean glucose concentrations, glucose variability, time in hyperglycaemia, time in range).<br>2. Mean nadir interstitial glucose levels, the risk of hypoglycaemia (assessed through the low blood glucose index), the risk of hyperglycaemia (assessed through the high blood glucose index), the number of HYPO episodes, the number of HYPO episodes (symptomatic and asymptomatic) and the number of HYPO |

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| | episodes requiring treatment for the 24-hour period following intervention completion.<br>3. The nadir, peak and postprandial blood glucose levels [assessed by the area under the curve (AUC)] and the peak and postprandial (assessed by AUC) insulin levels after a standardised lunch.<br>4. Future measurement of biochemical markers of appetite. |

## 2. ABBREVIATIONS

| | |
|---|---|
| AE | Adverse event |
| AEX | Aerobic Exercise |
| AR | Adverse reaction |
| CBG | Capillary Blood Glucose |
| CGM | Continuous Glucose Monitoring |
| CI | Chief Investigator |
| COVID-19 | Coronavirus disease 2019 |
| CRA | Clinical Research Associate (Monitor) |
| CRF | Case Report Form |
| CRO | Contract Research Organisation |
| CT | Clinical Trials |
| DM | Diabetes Mellitus |
| EC | Ethics Committee (see REC) |
| GCP | Good Clinical Practice |
| GP | General Practitioner |
| HYPO | Hypoglycaemia |
| ICF | Informed Consent Form |
| MMTT | Mixed Meal Tolerance Test |
| NHS | National Health Service |
| NRES | National Research Ethics Service |
| PHH | Postprandial Hyperinsulinaemic Hypoglycaemia |
| PI | Principal Investigator |
| PIS | Participant/ Patient Information /Sheet |
| 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 |
| SG | Sleeve Gastrectomy |
| SOP | Standard Operating Procedure |
| SUSAR | Suspected Unexpected Serious Adverse Reactions |
| TMF | Trial Master File |
| TTO | To Take Away |

## 3. BACKGROUND AND RATIONALE

Obesity is a major national and global public health challenge, which is associated with significant co-morbidities and increased mortality (1-4). In the United Kingdom (UK), more than 25% of the population is obese and approximately 10% suffers from severe and complex obesity (defined as BMI ≥35kg/$m^2$ with obesity related comorbidities) (5). Bariatric surgery is the most effective method to achieve substantial long-term weight loss and weight loss maintenance in patients with severe and complex obesity (6).

Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are the most commonly performed bariatric surgeries in UK and worldwide and accounts for more than 90% of UK bariatric procedures (7). Despite successful weight loss and weight loss maintenance, some long-term complications can develop after RYGB and SG, such as nutritional and vitamin deficiencies, dumping syndrome and postprandial hyperinsulinaemic hypoglycaemia (PHH) (4, 8, 9).

PHH is a common complication after RYGB and SG (8-10). PHH is characterised by low glucose levels and hypoglycaemic symptoms occurring 1-3 h after a meal (4, 8, 9). Data from continuous glucose monitoring (CGM) shows up to 70% of patients without type 2 diabetes (DM) spend 2% - 5% of their time with hypoglycaemia (HYPO) after bariatric surgery (interstitial glucose <3.0mmol/l) (11-13). Most of these episodes are asymptomatic (11); however, approximately 35% of those undergoing bariatric surgery will experience symptomatic PHH during daily life (14). Recurrent PHH after bariatric surgery is associated with reduced quality of life (10), weight regain (15) and may contribute to increased frequency of seizures, syncope and deaths due to accidents after bariatric surgery (16, 17).

The exact pathophysiology of PHH after bariatric surgery is unclear. However, it is believed that PHH is the result of the rapid arrival and absorption of a disproportionately large amount of carbohydrates at the distal small bowel postoperatively which triggers a corresponding over-secretion of insulin and gut hormones, contributing to glucose homeostasis (3, 4, 9, 18-21). Risk factors for developing PHH after bariatric surgery include being younger in age, normal glucose tolerance preoperatively, better pancreatic beta cell function preoperatively and higher insulin sensitivity (14, 22). Treatment options for PHH are limited, but dietary modification and management of carbohydrate intake is the first line treatment (2-4).

Exercise after bariatric surgery is a useful and complementary adjunct therapy as it elicits a multitude of beneficial health effects in these patients (23-29). More specifically, exercise after

bariatric surgery improves cardiorespiratory fitness (25), quality of life (22), physical function (26) and body composition (26, 27). It can mitigate weight regain and recurrence of obesity-related complications (27, 29). Despite the absence of evidence-based, bariatric surgery–specific physical activity recommendations, the European Guidelines for post-bariatric surgery management recommend that postoperatively, patients should adhere to a healthy lifestyle including moderate intensity aerobic exercise (AEX) for at least 30 minutes/day (30). However, recent evidence suggests that AEX after bariatric surgery in patients without DM enhances insulin sensitivity (24), a risk factor for PHH (22). Moreover, previous studies in people with DM have demonstrated that hypoglycaemia after AEX is relatively common in those using insulin or insulin secretagogues, as skeletal muscle insulin sensitivity is enhanced, especially during the first 24 hours following exercise, a mechanism that increases muscle glucose uptake and the subsequently the risk of hypoglycaemia in this population (31).

Currently, there is lack of evidence on the effect of an AEX bout on glucose levels and the risk of hypoglycaemia in people without diabetes who have undergone bariatric surgery. The proposed study will investigate whether a single bout of AEX increases the time spent in HYPO over the next 24-h in individuals without DM post-bariatric surgery . If this is the case, then guidance may be needed for prevention and management of PHH directly after AEX.

**Research Hypothesis**: We hypothesise that a single bout of moderate intensity AEX (in accordance with the European Guidelines for post-bariatric surgery management) in people without DM after bariatric surgery will increase the time spent in HYPO over the 24-h period after AEX.

# 4. OBJECTIVES

## 4.1 Primary Objectives

1) To investigate the effect of a single bout of aerobic exercise (AEX) on time spent in hypoglycaemia (HYPO) (defined as glucose levels <3.0mmol/l) and on glucose homeostasis during the first 24h after an AEX session.

## 4.2 Secondary Objectives

1) To assess the differences in CGM metrics (32) between the AEX and control (prolonged sitting) for the 24-h period after completion of the intervention [including CGM mean glucose concentrations, glucose variability, the time in hyperglycaemia and time in range (see section 5.2 for definitions of hyperglycaemia and time in range)].
2) To assess the difference between AEX and control condition in mean nadir interstitial glucose levels, the risk of hypoglycaemia (assessed through the low blood glucose index), the risk of hyperglycaemia (assessed through the high blood glucose index), the total number (symptomatic and asymptomatic) of HYPO episodes (defined as glucose levels <3.0mmol/l) during daily life and the number of symptomatic HYPO episodes requiring treatment for the 24-h period following intervention.
3) To assess the change in the nadir, peak and postprandial blood glucose levels [assessed by the area under the curve (AUC)] and the peak and postprandial (assessed by the AUC) insulin levels after a standardised lunch.

## 4.3 Exploratory End Point

1) Future measurement of biomarkers of appetite at the plasma samples.

# 5. STUDY DESIGN

## 5.1 Summary of Trial Design

This is a single centre, randomised, two-period, crossover study with the following experimental period sequences:

AEX intervention: prolonged sitting for ≈6 hours (h) 15 minutes (min) punctuated by 30min AEX performed at 60% $VO_2$peak

Control: prolonged sitting for ≈6h 45min

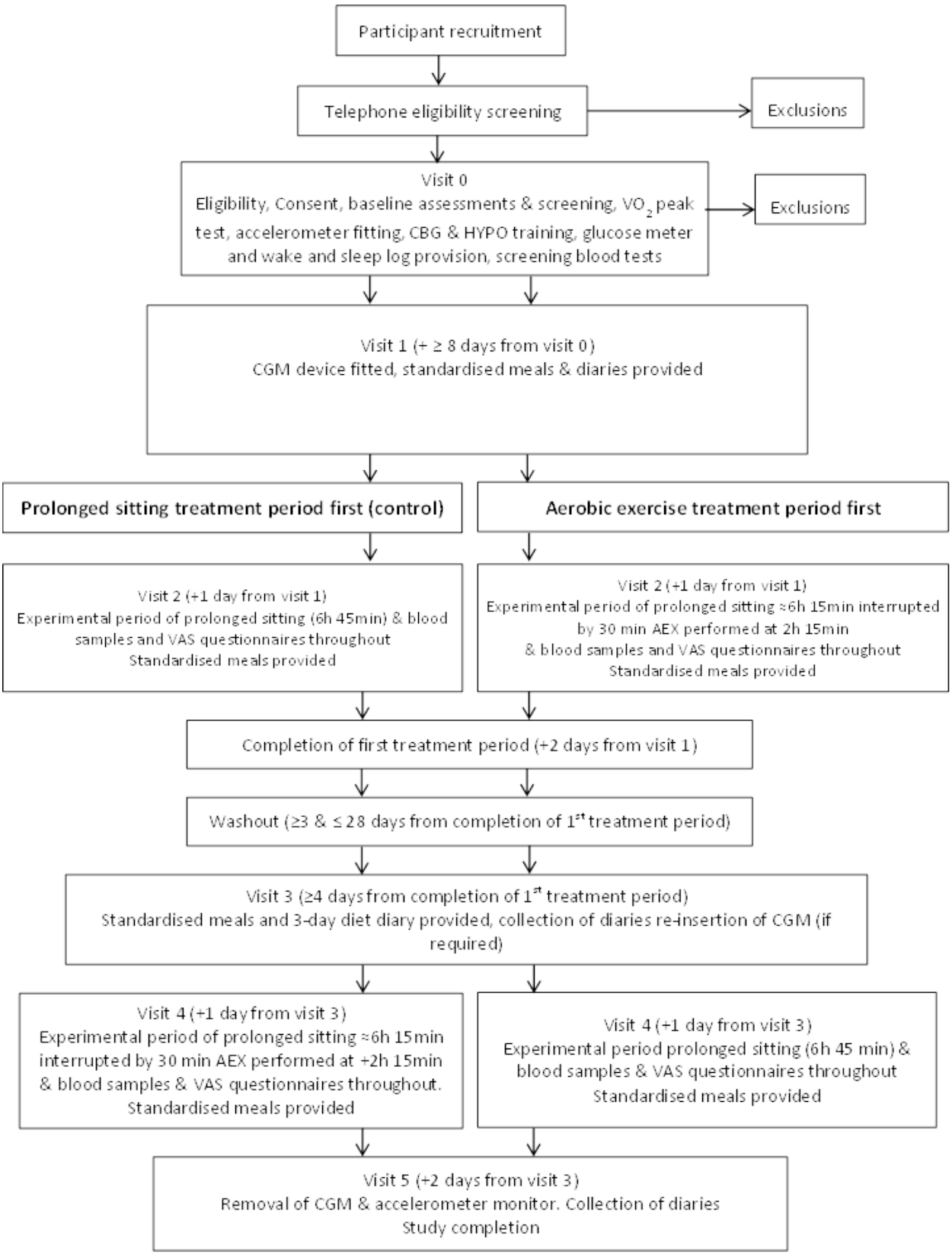
Group 1) Control at visit 2 and then intervention at visit 4

OR

Group 2) Intervention at visit 2 and then control at visit 4

The expected study duration is approximately one month per participant. Participants will attend six visits in total (Figure 1).

**Figure 1: Study flowchart**



The first visit (visit 0) is the screening visit and will occur a minimum of 8 days ahead of Visit 1. This visit 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. These samples will all be processed at the pathology laboratory within the Leicester General Hospital. A urine pregnancy test will also take place for all female participants of child bearing potential. 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. After screening and obtaining informed consent for study participation, eligible participants will undergo a progressive maximal exercise test on a treadmill with a 12 lead electrocardiogram and blood pressure monitoring. The maximal exercise test will be performed by an appropriately trained member of staff alongside a cardiac nurse or study clinician to ensure participants are safe for exercise participation and to determine their maximum aerobic capacity ($VO_{2peak}$). A blood glucose meter, brief training on capillary blood glucose (CBG) testing as well as on HYPO treatment in case of symptomatic HYPO, will be provided to all participants after the maximal exercise test (for safety purposes). Participants will keep the glucose meter for the whole study duration. Participants will also be given an accelerometer at visit 0 and asked to wear the accelerometer for the whole study duration (Visit 0 to Visit 5), however if they remove it for any reason, they will be given the wake and sleep log and asked to document the time and reason that the accelerometer was removed and the time that it was re-attached. The first eight days ahead of undertaking the first visit of the first treatment period (Visit 1) will measure habitual physical activity and provide information to ensure they are not over exerting themselves.

The study will consist of two treatment periods with duration of three consecutive days each and a 3 day minimum washout period between them (maximum washout period will be 28 days) (see Figure 1).

The study will consist of six visits in total. The experimental period (≈6h 45min duration) will take place on day 2 (Visit 2) of each treatment period and will include the intervention (30 min AEX bout or sitting in a chair) as well as a standardised lunch [mixed meal tolerance test (MMTT)] with frequent blood sampling (see figure 2). The impact of each intervention (30 min AEX bout or sitting in a chair) on the time spent in HYPO over the next 24-h will be assessed through continuous glucose monitoring (CGM, Dexcom G6) under standardised dietary, but otherwise free living conditions. The

two treatment (and experimental) periods will be identical; the only difference will be the intervention (30 min AEX bout or sitting). Participants will be asked to refrain from moderate-to-vigorous physical activity and alcohol for 24h prior to intervention visits. They will also be asked to standardise all food and drink during each of the 3-days treatment periods. This will be supported by recording all food and drink consumed (including time of consumption) using a 3-day diet diary (online or paper-based according to participant preference), with or without food-weighing scales (as preferred). Standardised meals will also be provided for the evening meal of day 1, breakfast, lunch and evening meal for day 2, and breakfast and lunch of day 3. Total energy content of meals will remain constant during the trial. Total macronutrient composition of standardised meals will approximate dietary reference values for the UK population [protein (15%), carbohydrate (50%) and fat (35%)]. A standardised snack with carbohydrates will be provided 15 minutes before the exercise to help avoid a symptomatic HYPO episode during the exercise. The snack will also be replicated in the prolonged sitting arm. Food preferences will be obtained from a pre-determined menu to guide standardised meals to be provided.

**At visit 1** those who successfully complete screening and the maximal exercise test will be randomised (1:1) to either experimental period sequence one or two (as described above).

**On day 1 for each treatment arm** (Visit 1 and Visit 3) a blinded CGM device (Dexcom G6) will be fitted to participants in addition to the accelerometer they will already be wearing (visit 1 only). The CGM will be worn continuously from visit 1 to visit 5. If the period between visit 1 and visit 5 is more than 10 days (which is the recommended use for the Dexcom G6 sensor), then a second CGM sensor (Dexcom G6) will be inserted at visit 3 for the second treatment period. If the window between visit 1 and visit 5 is less than 10 days then this visit 3 can occur remotely. A brief reminder will be provided to all participants in CBG testing in case of HYPO symptoms and in HYPO treatment and we will ensure that a blood glucose meter is still available to them from the screening visit. A glucose monitoring diary to record symptoms suggestive of hypoglycaemia, glucose levels at the time that they experience these symptoms and the treatment received for hypoglycaemia will also be provided to all participants at visit 1. Additionally, participants will be asked to complete a 3-day diet diary (as described above). Finally, all participants will be provided with a standardised meal from the research team to consume on the evening before each of Visits 2 and 4 (day 2 of each treatment period), no later than 10h before their study visit. Participants will be asked to fast and refrain from any food or drink, other than water (which may be consumed *ad libitum*), after

consumption of this standardised meal. Participants will be allowed to consume caffeinated drinks on the day before each of visits 2 and 4, but will be asked to record and replicate these as per any other food or drink item in both treatment periods, and not drink these after consumption of their standardised meal.

In a case where the participant will not need to attend visit 3 in person, all the above aspects will be covered in the phone call or video call with a participant at a time of visit 3.

Participants will be provided with a standardised evening meal to take and consume at a set time at home, as well as with a 3-day diet diary. This could be but is not limited to providing the meals and a diary at the previous visit or delivering the meals and a diary to the participant's home.

**Washout period**

The period between visit 2 and visit 3 is called a washout period and will start after completion of visit 2 and will last for min 3 to max 28 days. It is a period of time between the 2 treatment periods which is used to eliminate 'carry over' effects of the exercise on glucose levels. Participants will be asked to continue completing any of the diaries (glucose monitoring diary and wake and sleep log) throughout the washout period (between the 1st and 2nd treatment period), but this will not be obligatory.

**On day 2 of each treatment arm** (Visit 2 and Visit 4), after an overnight fast of at least 10h, participants will arrive at approximately 8:30am. Anthropometrics (weight, blood pressure and heart rate) will be measured on arrival. A cannula will be inserted; a blood sample for measurement of fasting glucose, insulin and appetite hormone levels (biomarkers of appetite) will be collected immediately before consumption of a standardised breakfast which will be provided at approximately 9am (allowing 15min for consumption).

Participants will be provided with a breakfast meal providing approximately 15% of their estimated daily energy requirements [estimated using Mifflin-St Jeor equation (MSJE) explained below and adjusted for habitual physical activity levels]. The duration of food consumption will be recorded to allow replication when individuals complete the crossover condition. After breakfast consumption the study clock will be started for the ≈6h 45min experimental period (+0h). After resting (sitting in a designated room equipped with a chair, desk, laptop and magazines), a standardised snack with carbohydrates will be provided at +2h (15 minutes before the intervention takes place) to help avoid severe hypoglycaemia during the AEX session (the snack will be replicated in the control session

also). The AEX or control intervention will be performed (based on randomisation) at +2h 15min in the exercise lab for 35min (until +2h 50 min). Blood samples for plasma glucose, insulin and appetite hormones will also be obtained through the cannula immediately before (+2h 15min) and immediately after (+2h 50min) the intervention.

The AEX session will be supervised by an appropriately trained member of the study team and conducted on a treadmill, at the same brisk walking speed self-selected during the maximal exercise test, at a gradient predicted to elicit 60% of each participant's $VO_2$ peak based on the screening assessment. The duration of the AEX session will be 30 min. This bout of AEX will be preceded by a 3-min warm up and followed by a 2-min cool down, such that the total bout will be 35 min. Blood pressure will be recorded before and after exercise and heart rate and rating of perceived exercise (RPE) will be recorded throughout. Participants will remain rested and seated throughout the control condition.

The participant will be provided a second meal (mixed meal tolerance test (MMTT)) approximately +3h 30min into the trial visit, comprising 30% of estimated energy requirements. The food items provided will be guided by participant preferences obtained at Visit 0. Meals consumed at Visit 2 will be replicated at Visit 4.

The MMTT will be consumed over 15min (extra 5 minutes will be given to complete meal consumption if needed) at visit 2 and 4. Blood samples (8 ml per sampling) for measurement of glucose homeostasis' parameters (glucose, insulin) will be collected at 11 time-points, through the inserted cannula, immediately before the meal (+3h 30min) and then 15min, 30min, 60min, 90min, 120min, 150min, 180min after lunch consumption. At the same time points additional blood sample of approximately 7ml per sampling will be taken for storage of plasma for future measurement of biomarkers of appetite (appetite hormones). Visual analogue scale (VAS) scores for hunger and satiety will be completed at each time point of blood sampling (30) (Appendix 2). After completion of the 3-h blood sampling period post-MMTT at lunchtime (≈+6h 45min, ~16:00), participants will be free to go home and resume their normal daily activities (see below for more detail).

Throughout the visit the study clinical team will monitor the participants for HYPO and the participants will also be asked to report any signs or symptoms suggestive of HYPO. If participants experience symptoms suggestive of HYPO, capillary/venous blood glucose will be measured at bedside through a glucose meter for safety purposes. Only symptomatic hypoglycaemia will be treated during the experimental period (visit 2). At the onset of a symptomatic HYPO (defined as

capillary/venous blood glucose <3.0 mmol/l with symptoms suggestive of HYPO), treatment will be provided in accordance with the University Hospitals of Leicester Hypoglycaemia Guideline.

If symptomatic HYPO takes place after completion of the intervention but before the initiation of the MMTT at lunchtime, then the HYPO will be treated and the standardised MMTT will take place but without sample collection for plasma glucose, insulin and biomarkers of appetite and VAS score for hunger and satiety. The participant will remain at sitting position after treatment of HYPO until the end of the experimental period at ≈+6h 45min. If symptomatic HYPO takes place after initiation of the MMTT at lunchtime, HYPO will be treated and the sample collection for plasma glucose, insulin and biomarkers of appetite as well as the VAS score for hunger and satiety will be terminated after treatment of HYPO. However, participant will remain at sitting position after treatment of HYPO until the end of the experimental period at ≈+6h 45min.

If symptomatic HYPO takes place before completion of the intervention, this will mean termination of the experimental period. The participant will be given the option to re-arrange this appointment in case of symptomatic hypoglycaemia before the completion of the intervention.

All participants' habitual physical activity will be assessed using a wrist-worn accelerometer. Participants will be asked to test their CBG levels only if they experience HYPO symptoms and to treat HYPO only if CBG <3.0mmol/l. It is expected that 80% of HYPO episodes will be asymptomatic (8).

Participants will be provided with a standardised evening meal to take and consume at a set time at home, as well as with a standardised breakfast and lunch for the following day (day 3 of each investigational period) to be consumed at set times.

Participants will be asked to keep a 3-day diet diary, glucose monitoring diary and a wake and sleep log until 24-h after the end of the intervention. Participants will also be encouraged, but this will not be obligatory, to continue keeping a glucose monitoring diary and wake and sleep log during the washout period of the study (between the 1$^{st}$ and 2$^{nd}$ treatment period), as they will continue to wear an accelerometer and the continuous glucose monitoring device (until at least the end of sensor's life for the CGM device).

**On day 3 of the second investigational period** (Visit 5) participants will arrive at least 24-h after completion of the intervention (after midday) for accelerometer and CGM removal as well as returning the glucose monitoring diary, the 3-day diet diary, the glucose meter and strips and the

wake and sleep log. If the participant prefers to post the monitors and diaries back, this will be an option.

## 5.2 Outcome Measures

All clinical measures will be carried out in accordance with the Sponsor's, the University Hospitals of Leicester (UHL), the Leicester Diabetes Centre, the Clinical Trials Unit and study specific standard operating procedures (SOPs).

Table 1: Data collection (including screening measurements); primary and secondary outcome measures, when they are being assessed, and who will be obtaining the measurements.

| | Measurement type | Measurement (Units) | Assessment measured | Who is recording/ conducting the measurement? |
|---|---|---|---|---|
| **Screening data collection** | **Demographic data** | Date of birth<br>Age<br>Gender<br>Ethnicity<br>Socioeconomic status | Baseline – Visit 0 | An appropriately trained member of the study team |
| | | Smoking status<br>Alcohol status<br>Employment status | Baseline – Visit 0 | |
| | **Medical history** | Date of bariatric procedure<br>Months since procedure | Baseline – Visit 0 | An appropriately trained member of the study team |
| | | Medical history & medications | Baseline – Visit 0 | |
| | **Aerobic fitness** | VO2 peak – exercising ECG | Baseline – Visit 0 | An appropriately trained member of the study team will perform the maximal exercise test alongside a cardiac nurse/clinician who will be overseeing the ECG and monitoring the participant. |
| | **Estimated daily energy requirements** | Mifflin-St Jeor equation (MSJE) | Baseline – Visit 0 | An appropriately trained member of study team |

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| | **Anthropometric** | Height (cm) | Baseline – Visit 0 | An appropriately trained member of the study team |
| | | Body mass (kg) | Baseline – Visit 0, 2 and 4 | An appropriately trained member of the study team |
| | | Body mass index (kg/m$^2$) | Baseline – Visit 0, 2 and 4 | An appropriately trained member of the study team |
| | | Waist circumference (cm) | Baseline – Visit 0, 2 and 4 | An appropriately trained member of the study team |
| | **Cardiovascular measures** | Blood pressure (mmHg) & resting heart rate (bpm) | Visit 0, 2 and visit 4 - Multiple time points | An appropriately trained member of the study team |
| | **Physical activity** | Accelerometer [*mg*]) | Visit 0 – 5 | A trained member of the study team will instruct the participant on how to wear the accelerometer and explain how to fill in the wake and sleep log |
| | **Blood samples** | Full blood count (FBC)<br>Renal function test (U+Es, eGFR)<br>Liver function test (LFTs)<br>HbA1c | Baseline – Visit 0 | An appropriately trained member of the study team |
| **Primary Outcome Measure** | **Glucose levels** | Interstitial glucose levels ( Hypo; <3.0mmol/l) through Dexcom G6 | From approximately<br>midday visit 2 for 24 h | An appropriately trained member of the study team |

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|---|---|---|---|---|
| | | | midday visit 5 for 24 h | |
| **Secondary Outcome Measures** | **Continuous glucose monitoring outcomes** | Mean Glucose concentration | From approximately midday visit 2 for 24h<br>From midday visit 5 for 24h<br>(and continuous monitor wear from visit 1 to 5) | An appropriately trained member of the study team |
| | | Glucose variability (CV% and SD) | | |
| | | Duration of hyperglycaemia:<br>Level 1: Glucose levels >7.8mmol/l (% time)<br>Level 2: Glucose levels >10mmol/l (% time) | | |
| | | Differences in mean nadir interstitial glucose levels<br>Glucose levels <3.9mmol/l (% time)<br>Glucose levels <3.3mmol/l (% time)<br>Glucose levels <2.2mmol/l (% time) | | |
| | | Number of episodes of HYPO (glucose levels<3.0mmol/l)<br>Number of episodes with glucose levels <3.3mmol/l<br>Duration of each hypoglycaemic episode (minutes, % time) | | |
| | | Risk of hyperglycaemia [assessed by high blood glucose index (HBGI)]<br>Risk of hypoglycaemia [assessed by low blood glucose index (LBGI)] | | |

| | | | | |
|---|---|---|---|---|
| | | Number of episodes requiring HYPO treatment for the 24-h period following completion of each intervention | | |
| | | Time in range:<br>Definition: 1. Interstitial glucose levels 3.9 – 7.8mmol/l (% time)<br>Definition 2. Interstitial glucose levels between 3.0 and 7.8mmol/l (% time) | | |
| | | Glucose levels between 3.9 -10mmol/l (% time)<br>Glucose levels between 3.0 -10mmol/l (% time) | | |
| | **Blood glucose & insulin at the mixed meal tolerance test (MMTT)** | Pre-MMTT, peak, nadir (nadir levels only for glucose) and time-averaged postprandial area under the curve (AUC) for the MMTT (lunch-time) | Visit 2 and Visit 4- Multiple time points | An appropriately trained member of the study team |
| | **Other blood glucose and insulin measurements** | Fasting, immediately before and immediately after exercise | Visit 2 and Visit 4 – Multiple time points | An appropriately trained member of the study team |
| | **Questionnaires** | Visual analogue Scale (VAS) score - VAS hunger and satiety score | Visit 2 and Visit 4 – Multiple time points – Calculated from AUC | Participant self-assessment completion |
| **Exploratory end point** | **Plasma Samples** | Appetite hormones (biomarkers of appetite) | Visit 2 and visit 4- Multiple time points | An appropriately trained member of the study team |

**Primary outcome**

1. Difference in % time in hypoglycaemia (defined as interstitial glucose levels <3.0mmol/l) in continuous glucose monitoring between the two interventions (AEX vs control) after bariatric surgery during the 24-h period after completion of the intervention.

**Secondary outcomes**:

1. Difference in Area Under the Curve $(AUC)_{(0\text{-}180)}$, pre-meal, peak and nadir glucose levels after mixed meal tolerance test (3-h MMTT) between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
2. Difference in $AUC_{(0\text{-}180)}$ insulin, pre-meal and peak insulin levels during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
3. Difference in the ratio $AUC_{(0\text{-}180)}$ insulin/$AUC_{(0\text{-}180)}$ glucose during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
4. Difference in the ratio $AUC_{(0\text{-}30)}$ insulin/$AUC_{(0\text{-}30)}$ glucose during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
5. Difference in the ratio $AUC_{(60\text{-}180)}$ insulin/$AUC_{(60\text{-}180)}$ glucose during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
6. Difference in the ratio of maximum/minimum plasma glucose during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
7. Difference at the number of mixed meal tests required to be stopped due to symptomatic HYPO with blood glucose/capillary glucose levels <3.0 mmol/l during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).
8. Difference in %time in interstitial glucose levels <3.9 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.
9. Difference in %time in interstitial glucose levels <3.3 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.
10. Difference in % time in interstitial glucose levels <2.2 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

11. Difference in % time in range (definition 1, defined as interstitial glucose levels between 3.9 – 7.8 mmol/l) in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

12. Difference in % time in range (definition 2, defined as interstitial glucose levels between 3.0 – 7.8 mmol/l) in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

13. Difference in % time in interstitial glucose levels between 3.9 – 10 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

14. Difference in % time in interstitial glucose levels between 3.0 – 10 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

15. Difference in % time interstitial glucose >7.8 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

16. Difference in % time interstitial glucose >10 mmol/l in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

17. Difference in the mean interstitial glucose in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

18. Difference in the standard deviation (SD) of the mean interstitial glucose between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

19. Difference in the coefficient of variation (CV) (CV=SD/mean interstitial glucose) between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

20. Difference in the frequency and intensity of symptoms suggestive of postprandial hypoglycaemia reported by the patients during CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

21. Difference in the number of hypoglycaemic events/day (defined as interstitial glucose levels <3.0 mmol/l) in CGM between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

22. Difference in the number of hypoglycaemic events/day (defined as interstitial glucose levels <3.0 mmol/l) in CGM with symptoms suggestive of hypoglycaemia required treatment between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) during the 24-h period after completion of the intervention.

23. Difference in risk of hyperglycaemia between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) calculated as the high blood glucose index (HBGI) for the 24-h period after completion of the intervention using the EasyGV workbook (www.phc.ox.ac.uk/research/technology-outputs/easygv).

24. Difference in risk of hypoglycaemia between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG) calculated as the low blood glucose index (LBGI) for the 24-h period after completion of the intervention using the EasyGV workbook (www.phc.ox.ac.uk/research/technology-outputs/easygv).

25. Difference in $AUC_{(0\text{-}180)}$ satiety and hunger Visual Analogue Scale levels during the 3-h MMTT between the two interventions (AEX vs control) after bariatric surgery (RYGB and SG).

# 6. TRIAL PARTICIPANTS

## 6.1 Overall Description of Trial Participants

Patients will be recruited via three routes, within primary and secondary care, previous study participants and community settings. In the UK, after bariatric surgery, patients remain under secondary care for the first two postoperative years, after which they are discharged to primary care. Post-bariatric surgery clinics at the University Hospitals of Leicester (UHL) will be used for recruitment. A database with all the patients who have undergone RYGB and SG at UHL over the last 15 years (~500 patients) is available and will be used for identification of eligible participants in primary care. Participants whom have undertaken previous studies locally and consented to future research will also be invited. Community settings may include recruitment of people at community/community events and meetings, recruitment/health fairs in community, study advertisement (e.g. posters, leaflets and social media) and use of Research Registries (including but not limited to the Leicester Research Registry).

Male and female participants aged ≥18 and <75 years and at least ≥12 months after bariatric surgery (RYGB or SG) will be invited to participate in the study. A total of 18 participants will be recruited to the study accounting for a 20% drop out. Recruitment strategies will include identifying participants who meet the following inclusion/exclusion criteria. Potential participants will receive a short participant information sheet and response slip which can be returned if interested in participating. Upon receipt of the expression of interest reply slip, telephone screening of the inclusion and exclusion criteria will be undertaken.

## 6.2 Inclusion Criteria

1. Aged ≥18 years but less than 75 years
2. Subjects ≥1 year after gastric bypass (RYGB) or sleeve gastrectomy (SG)
3. Able to understand written and spoken English
4. Able to provide informed consent

## 6.3 Exclusion Criteria

1. A 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)

2. Diagnosis of type 1 diabetes
3. An eGFR value of ≤30 ml/min at screening blood tests
4. History of revisional bariatric surgery, except of previous gastric banding which has been removed
5. Any other bariatric operation which is not RYGB (single anastomosis bypass patients will be excluded) or SG
6. On any medications that affect glucose levels (e.g. glucose-lowering medications or systemic corticosteroids – not including use of topical or inhaled corticosteroids)
7. Any contraindications/limitations to AEX self-reported or identified in the maximal exercise test (e.g orthopaedic limitations, severe cardiovascular/pulmonary disease, chair bound)
8. An established diagnosis of PHH
9. Systolic blood pressure ≥160mmHg or Diastolic blood pressure ≥100mmHg at screening
10. Individuals which are taking part in regular structured exercise
11. Being on acarbose, diazoxide, octreotide or other treatment for postprandial hypoglycaemia
12. History of epilepsy
13. HbA1C ≥ 6.5% or ≥48mmol/l at screening blood tests
14. Haemoglobin (Hb) <100 g/L at screening blood tests
15. Currently pregnant or breastfeeding
16. Recent active infection (over the last 10 days)
17. Adrenal insufficiency and/or substitution with glucocorticoids
18. >180kg at screening due to the maximum weight capacity of the treadmill
19. Participating in another research study involving intervention within 3 months of screening
20. Severe intolerance to the standardised meals and Mixed Meal Tolerance Test (MMTT) – severity of intolerance to be assessed by a clinician during screening visit

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

See figure 1 for the study flow diagram. There will be a total of six visits.

Visit 0 - screening and eligibility visit, the following will take place at this visit: baseline assessment, baseline questionnaires, screening blood tests, maximal exercise test, accelerometer and wake and sleep log provision, CBG test training and HYPO treatment training. All female participants of child bearing potential will have urine pregnancy test.

At Visit 1, those who successfully complete screening and the maximal exercise test will be randomised (1:1) to either experimental period sequence one or two, below:

1. Prolonged sitting for ≈6h 45min (control) followed by sitting [total ≈6h 15min] and one bout of AEX for 30min

OR

2. Sitting (total ≈6h 15min) and one bout of AEX for 30min followed by prolonged sitting for ≈6h 45min (control).

Visit 1 and 3, this is the first visit in each treatment period [device fitting (CGM), diaries provision (3-day diet diary and blood glucose monitoring diary) and standardised meal provision]. 3-day diet diary from the first treatment period will be collected at visit 3 and a new one will be issued. Participants will have an option to return the diaries by post or on the next face to face visit.

However, if there are issues regarding sensor life (for example if the end of the second treatment period is more than 10 days from Dexcom G6 sensor insertion), a new CGM sensor will be inserted at visit 3 by an appropriate member of the study team; a new glucose monitoring diary with be issued. Moreover, Visit 3 could be optional to attend in case where CGM devise does not need to be re-inserted (if <10 days between insertion of CGM device and end of treatment period 2 and participant will continue wearing the CGM) and standardised meals and new set of diaries and logs may be provided as required in advance of this visit. In such case visit 3 can be carried out over the phone or video call as per participant's preference.

However, as described above, should patient require CGM device to be re-inserted at visit 3, they would need to attend Leicester Diabetes Centre for this visit.

Visit 2 and 4 include the experimental period (blood samples and the ≈6h 45min of either sitting or 6h 15min sitting plus 30 min of AEX with standardised meals]; VAS score will be completed at the time points of blood samples being drawn.

Visit 5 – Approximately 24h after visit 4 (midday visit 5), devices removal (accelerometer and CGM) and collection of 3-day diet and blood glucose monitoring diaries and wake and sleep logs will occur. Devices removal at visit 5 (if device removal takes place at Leicester Diabetes Centre) will be performed by an appropriate member of the study team.

If the participant prefers to remove the CGM device themselves and post the monitors and diaries back, this will be an option after completion of the first and second treatment period. Therefore Visit 5 could be an optional visit to attend and can be carried out over the phone or video call as per participant's preference.

### 7.1 Recruitment and Informed Consent

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 and West Midlands Clinical Research Network. Recruitment will commence once ethical and regulatory approval has been granted and Sponsor green light given. A team of four consultants in Upper Gastrointestinal (UGI) surgery provide bariatric surgery to the local population (Leicester /Leicestershire/ Rutland) in University Hospitals of Leicester NHS Trust. The main recruitment avenues will be secondary care bariatric surgery clinics (Tier 4 follow-up clinics) and database lists of previous patients at the University Hospitals of Leicester NHS Trust, private practice clinics (surgical clinics) and primary care (GP Practices) for individuals who have been discharged from Tier 4 service. For this study, 18 patients who have undergone RYGB or SG will be recruited (for details in study power calculation please see section 11.2 The Number of Participants). Over the last 10 years, more than 500 patients have been operated with RYGB or SG 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). The direct healthcare team will undertake the necessary database searches based on the study recruitment criteria, then send or provide directly eligible participants with a pack comprising an invite letter, a short version of the participant information sheet (PIS) and reply slip. Follow-up calls will also be provided two weeks after the initial mailing. Opportunistic marketing

will also be used, e.g. leaflets and posters, displayed in appropriate community hubs. The planned recruitment target is 1 to 2 participants per month.

Due to the current global pandemic we will not encourage participants to attend or take part if it poses a risk to them. We will encourage participants to attend visits by themselves to reduce people coming into the Leicester Diabetes Centre. Participants will also undergo a COVID-19 check which includes a temperature check and some questions and if participants are able to they must wear a face mask/covering when they attend their appointment as per National Government Guidance.

The risk of COVID-19 infection will be minimised by staff wearing PPE and following National Government Guidance and having COVID-19 Risk Assessments in place to reduce risk of infection.

Potential recruitment activities include;

1. Primary care

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:

    Surgical team and dieticians will make the initial approach to eligible patients and will provide them with leaflet.

        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. Attendance of surgical private practice outpatient clinics

3. Previous research participants – At the Leicester Diabetes Centre, approximately 50 adults who have undergone bariatric surgery have previously been screened for the "MOTION" study. Those eligible patients who have consented to being contacted regarding future ethically approved research will be contacted.
4. Community/community events and meetings
   a. Pharmacists, GP mentors, other Healthcare professionals and community workers
   b. Via community health fairs – Study team would participate in these events to publicise the study and distribute information. This could consist of having a stand with all the study information and/or presenting the study event dependant. Due to the current COVID-19 situation, study team may not be able to participate or hold face to face meetings. Study team will only participate in community events if possible, if participating in community events study team will ensure they follow COVID-19 National Governance Guidance and any other measures in place to ensure safety of themselves and others. Alternatively study team can circulate study poster and other supporting documents to key people within the community to advertise study on community websites to minimise Face to Face contact.
5. 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. Use of Research Registries to advertise the study, including but not limited to the Leicester Research Registry.

### 7.2 Screening and informed consent

Once the potential participant has expressed an interest (e.g. returned the reply slip or called) the BariEX team will contact to undertake the telephone screening to ensure they are not invited to join the study if they are ineligible.

During the telephone screening, the non-measured inclusion and exclusion criteria will be checked (reported in section 6.2 and 6.3). The telephone screening will provide an opportunity to discuss the study and ask any questions directly to a member of the research team. We do not encourage participants to attend or take part in the study if they are putting themselves at risk by attending hospital.

Upon completion of the telephone screening phase, potential participants will be invited to attend an initial appointment (Visit 0) to obtain consent, undertake screening measurements and collect baseline data (including screening bloods). A letter will be sent to confirm this appointment along with the full participant information sheet (PIS). Study documents will be sent to potential participants to allow them at least 24h to read and consider the information, giving them the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. The study team will record when the PIS has been sent and this will be at least five working days before a study visit. Visits will take place at University Hospitals of Leicester premises where appropriate resuscitation facilities are available.

For all study visits that participants will attend the Leicester Diabetes Centre, Research staff at Leicester Diabetes Centre will follow and adhere to Public Health and NHS Trust Guidance and organizational assurance risk assessments and standard operating procedures that are in place due to COVID-19 to ensure the safety of participants and staff and to minimise the risk of COVID-19 infection. 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 deliver the study in a safe manner.

At the initial appointment, eligibility will be confirmed and informed consent will be taken in line with good clinical practice by a trained, delegated member of the research team. Consent will be taken prior to any study procedures being carried out. Written informed consent will be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The original signed form will be retained within the Trial Master File

(TMF). A copy of the signed informed consent will be given to the participant and a further copy retained in the participant's medical notes along with a copy of the PIS.

Consent will be carried out face to face, (due to COVID-19) risk assessments have been carried out and put in place in rooms where consent will be taken. Research staff will need to adhere to guidelines put in place when taking consent. They will need to ensure suitable sized rooms are used to allow proper social distancing. Use of hand wash, alcohol hand sanitizer, and appropriate use of PPE. Research staff will also need to clean down areas after use. Participants will be encouraged to bring their own pens for their consenting visit.

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 case report form (CRF) by a member of the study team.

All participants are free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

A recruitment target of 1 to 2 participants per month over the two year study period will allow the investigators to meet the study objectives and is within the resource capacity of the team.

### 7.3 Collection of baseline data

After taking consent, the following demographic and clinical information and outcome measures will be obtained (Visit 0):

**Demographic data and medical history (Visit 0)**

The study team will collect the following demographic and medical history data and record it in the CRF: date of birth, age, gender, ethnic background, socioeconomic status (derived from postcode), smoking status, alcohol status, employment status and associated details of their bariatric surgery procedure, any relevant history of disease, prescribed and concomitant medications, or any other relevant surgical interventions.

**Physical examination (Visit 0)**

The following anthropometric measures will be obtained: Height (cm), Waist circumference (cm), body Mass (kg) body mass index ($kg.m^2$). Systolic and diastolic blood pressure (mmHg) and resting heart rate (bpm) will be recorded after the participant has been seated for at least five minutes. An accelerometer will be fitted ahead of the maximal exercise test.

**Screening blood tests (Visit 0)**

Blood tests for screening for diabetes (HbA1C), haemoglobin levels (Full blood count, FBC), kidney (U+Es and eGFR) and liver function (LFTs) will be performed at visit 0. All blood tests performed during the study will be reviewed and the reports signed by the Investigator (or an individual/ individuals approved by the CI as listed on the delegation of authority log) who will record in the CRF whether they are normal, abnormal but not clinically significant, or abnormal. If abnormal a letter will be sent to the GP.

**Estimation of Daily Energy Requirements (Visit 0)**

The daily energy requirements for each participant (for provision of the standardised meals to participants during the treatment periods in accordance with their daily energy requirements) will be estimated using the Mifflin-St Jeor equation (MSJE) which uses height, weight and age to calculate resting energy expenditure. The activity factor will depend on level of activity categorised as sedentary, lightly active, moderately active, very active and extra active.

**Maximal exercise test and ECG (Visit 0)**

The supervised maximal exercise test is a reliable measurement of physical capacity and aerobic fitness (33, 34), with a 12 lead electrocardiogram, blood pressure monitoring and other associated measures. The test will be used as a screening measure in order to ensure that participants are safe to participate in exercise and to determine their maximum aerobic capacity ($VO_2$peak) which will inform the exercise level performed on visits 2 or 4. This incremental walking test will identify potential contraindications to exercise and will assess the suitability to participate to the study. The supervised maximal exercise test report will be signed by the delegated investigator who will record in the CRF whether it is normal, abnormal but not clinically significant, or abnormal AND clinically

significant. Referrals to the appropriate healthcare professional, i.e. cardiology or the participants' general practitioner (GP) will be made in any such case. The participant may be retained and re-evaluated if no issues of concern emerge; otherwise, the participant will be withdrawn. A blood glucose meter and training on capillary blood glucose (CBG) testing and HYPO treatment in case of symptomatic HYPO will be provided to all participants after the maximal exercise test at visit 0 (for safety purposes).

A graded treadmill maximal exercise test will be undertaken at a fixed speed (as determined by participant ability) with progressively increasing gradient, according to SOPs developed within the NIHR Leicester Biomedical Research Centre. The test includes participant pre-screening involving medical history review by an appropriately trained member of the study team and resting electrocardiography (ECG).

After an opportunity to familiarise themselves with the treadmill, participants will complete a 3-5 minute warm up at a light intensity (speed ≤4km/h). Participants will then walk at the fixed speed at a 0% gradient, before the gradient increases by 1% each minute for the duration of the test. A walking speed of less than 4km/h will be selected where participants are unable to comfortably walk at this pace without incline. Participants will be encouraged to continue the test until (a) volitional exhaustion, (b) participants reached 100% of their age-predicted maximum heart rate (85% if taking β-blockers) and a Respiratory Exchange Ratio (RER) of ≥ 1.15 or (c) the test is terminated by the cardiac nurse/clinician, who will monitor the entire test using a 12-lead ECG. The latter will be classified as an incomplete test. In the instance where cardiac nurse/clinician suspects a cardiac abnormality and terminates the test, the participant will be referred for further investigation

An appropriately trained member of staff will assess heart rate and collect expired air that will be processed through an automated breath-by-breath analyser. Rating of perceived exertion (RPE) (rating 6-20) will also be recorded regularly throughout the test. A rolling average of 10 breaths will be calculated for $\dot{V}O_2$ and $\dot{V}CO_2$, and peak oxygen uptake ($\dot{V}O_{2peak}$) will be defined at the highest number. The RER and heart rate at $\dot{V}O2_{peak}$ will also be reported.

### 7.4 Primary outcome measure

**Continuous glucose monitoring (CGM) (Dexcom G6) (Visits 1 and 3)**

CGM is a minimally invasive technique used for monitoring interstitial blood glucose levels. A commercially available glucose sensor (Dexcom G6) is inserted into the abdominal subcutaneous tissue (5mm depth) and measures glucose levels at this site continuously for up to 10 consecutive days (duration of life of a sensor). It provides an average blood glucose reading every five minutes and is suitable for use during normal everyday activities including bathing and most physical activities. The device does not require calibration with CBG monitoring, however the CGM receiver should be within a 6 metre distance of the CGM transmitter in order to capture all CGM recordings. We anticipate obtaining more than 20h of stable recording over the 24h period of monitoring post-intervention for each treatment period (primary outcome). The CGM data will be blinded to ensure patients' diet habits are not affected by monitoring of glucose levels. When the device is detached, the software uploads time stamped data in an excel format.

At visit 1 the blinded CGM device (Dexcom G6) will be fitted to participants and a glucose monitoring diary provided to monitor throughout the treatment period timeframes. Full training in CBG testing in case of HYPO symptoms and education on HYPO treatment will be provided to all participants at visit 0 with education reminders at visits 1 and 3, before returning home. Participants will use the same blinded CGM device throughout the study period. However, if there are issues regarding sensor life (for example if the end of the second treatment period is more than 10 days from Dexcom G6 sensor insertion), a new CGM sensor will be issued at visit 3 where necessary, together with a new glucose monitoring diary.

### 7.5 Secondary outcome measures

#### Blood samples for insulin, glucose and appetite biomarkers

Blood sample collection (insulin, glucose and biomarkers of appetite) throughout the experimental period and during lunch time mixed meal tolerance test (MMTT) will be undertaken. The MMTT will be performed 45mins after completion of AEX and at a similar point in the control condition) (Visits 2 and 4).

Glucose will be measured at 11 time points throughout the duration of each experimental period using cannulation of the participant's forearm. The blood samples will be collected at the following time points: when the participant arrives in a fasted condition, immediately before the intervention (+2h 15min), immediately after the end of the intervention (+2h 50min), immediately before the MMTT at lunchtime (+3h 30min) and then after completion of lunch at 15 minutes, 30 minutes, 60

minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes. Consumption of the standardised MMTT at lunchtime will be completed within 15 minutes (extra 5 minutes will be given to complete meal consumption if needed). A total of 88ml of blood (approximately 8ml per sample) will be collected for insulin and glucose.

An optional extra amount of approximately 7 mls per sampling (overall additional 77 mls of blood) will be collected for sample storing for future analysis of appetite biomarkers (if consent has been obtained). These samples will be analysed in a specialised lab based at Loughborough University.

Blood for glucose levels will be collected into Fluoride/Oxalate tubes. Glucose samples will be measured using standardised quality-controlled assays at the University Hospitals of Leicester NHS trust.

Blood for measurement of insulin will be assessed in fasting condition, pre-MMTT (i.e., immediately before the standardised MMTT at lunchtime) and then starting from the 'time of the last mouthful of the meal' at 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes post-meal 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 for insulin will be kept on ice prior to their use.

Blood samples for majority of appetite biomarkers will be collected into pre-cooled blood collection tubes pre-treated with 250ul of aprotinin (EDTA 4.9ml, 1 tube at each time point) at the same time points that blood sampling for insulin and glucose takes place. At the same time points, 2ml of blood will be collected with syringe and will be processed in accordance with the Leicester Diabetes Centre standard operating procedures for blood collection for measurement of acylated ghrelin, a hunger hormone.

**Hypoglycaemia (HYPO) during the MMTT**

This will be defined as plasma glucose ≤3.0mmol/l during the 3-h MMTT (please see section 9.4 for definitions of HYPO during MMTT).

It is noted that decisions regarding treatment of HYPO 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 the participant experiences symptoms suggestive of HYPO and venous blood glucose/capillary blood glucose levels

are ≤3.0mmol/l, the participant will receive treatment for HYPO in accordance with HYPO Guideline for Adults of the UHL. The blood collection for measurement of insulin, glucose and appetite biomarkers as well as the VAS score collection for hunger and satiety during the MMTT will be terminated. The participant will remain in sitting position after HYPO treatment until the expected time of completion of the experimental period (≈+6h 45min). It is not expected that patients will experience severe hypoglycaemia during the study as patients will be excluded at screening if they report frequent and severe symptoms of postprandial hypoglycaemia during daily life or they have an established diagnosis of PHH after bariatric surgery.

### 7.6 Reporting Procedures

***Ambulatory Activity - Accelerometer (Visit 0 to visit 5)***

Participants will be given an accelerometer at visit 0 and asked to wear the accelerometer for the whole study duration (Visit 0 to visit 5). The first eight days ahead of undertaking visit 1 (initiation of first treatment period) will measure habitual physical activity. The accelerometer will record sleep duration and quality, overall daily physical activity volume, time spent sedentary and time spent in light and moderate to vigorous physical activities (MVPA). Data will be used to assess habitual lifestyle behaviours and to assess compliance with exercise restrictions (no MVPA) prior to each clinical visit. Participants will also be asked to complete a wake and sleep log during the study. If participants remove the accelerometer, they will be asked to document the time that they removed it, the reason for it and when they re-attached it. An appropriately trained individual will instruct on correct placement of the monitor using the normal operating procedures. Participants will be given a stamped addressed envelope to return the accelerometer via post after the end of the second treatment period (if they prefer not to attend visit 5 in order to bring the accelerometer personally to Leicester Diabetes Centre). Data will be processed using an open source R programme (GGIR) according to SOPs developed for the NIHR Leicester Biomedical Research Centre.

***Questionnaire Data***

**Satiety and Hunger VAS score -** these scores will assess the feeling of satiety and hunger during the MMTT test at visits 2 and 4 [before initiation of the MMTT and 15', 30', 60', 90', 120', 150' and 180'

after consumption of the last mouthful of the meal)]. This will be completed at the time points above once blood samples have been drawn.

Additionally, at Visit 0 participant's self-reported physical activity level will be assessed using the International Physical Activity Questionnaire Short Form (IPAQ-SF).

**Diet (food and drink)**

Dietary intake and compliance with the standardised meals, along with times of food and drink consumption will be self-reported via a 3-day diet diary recorded over the two treatment periods. Pseudonymised data will be entered into a specialist dietary analysis software package (Nutritics). Intake will be analysed for total daily energy intake and macronutrient composition (carbohydrate, fat and protein). Compliance to standardised meals will be monitored and non-compliance recorded.

**Episodes of hypoglycaemia during visits and daily life**

All participants will be provided with a glucose monitor and a glucose monitoring diary during the study in order to be able to check their capillary glucose levels in case they experience symptoms suggestive of HYPO. If participants lose their glucose monitor during the study, a replacement monitor will be provided to them by the study team. Details on how to manage symptomatic hypoglycaemia will also be provided to the patients at visit 0. Definition of hypoglycaemic episodes as well as the different categories of hypoglycaemia is described below at section 9.4. Patients will be asked to document in the glucose monitoring diary any symptoms suggestive of HYPO during their visits and daily life. Patients will be asked to check their capillary glucose levels at the time that they experience symptoms and to document these in their glucose monitoring diary with details of the time, the date, and symptoms they experience. Participants will be asked to treat HYPO episodes only if glucose levels <3.0 mmol/l. Participants that are diagnosed with HYPO during the study will be provided with a blood glucose meter to keep after completion of the study. In the event that they lose their glucose meter after the end of the study, they would be required to speak to their G.P in order to recommend or replace it with a new one. Participants diagnosed with PHH will also be provided with an information leaflet on how to modify their diet at the end of the study. If symptoms of PHH are not improving with diet modifications, participants will be asked to consult their G.P on treatment options available to them to manage their symptoms.

**Biochemical analysis – Blood Samples (screening, visits 2 and 4)**

All laboratory results of blood tests performed during the study will be reviewed and the reports signed by the Investigator (or an individual/ individuals approved by the CI as listed on the delegation of authority log) who will record in the CRF whether they are normal, abnormal but not clinically significant, or abnormal. If abnormal a letter will be sent to the GP.

**Laboratory Tests/ biochemical analysis (screening, visits 2 and 4)**

Blood for measurement of FBC, renal function, liver function and HbA1C will be obtained at screening visit and will be analysed in UHL accredited laboratories and destroyed once analysed in accordance with the UHL's SOPs.

Blood for the measurement of glucose, insulin, and plasma for future analysis of appetite biomarkers (if consent is obtained for appetite biomarkers measurement from participant) will be obtained in fasting condition, immediately before and immediately after the intervention and during the 3-h standardised MMTT at 8 time points during each visit [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].

Blood glucose samples will be analysed in UHL accredited laboratories and destroyed once analysed in accordance with the UHL's SOPs. All laboratory results will be reviewed and the reports signed by the Investigator (or an individual/ individuals approved by the CI as listed on the delegation of authority log).

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

Once blood samples for insulin 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. Samples for measurement of insulin 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 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 sample's life to ensure that they will be used following the conditions that participants have given consent for.

**Exploratory endpoint**

The feasibility for the measurement of biomarkers of appetite (such as gut hormones) will be determined as new knowledge. Participants will have the option to consent to provision and storage of their samples for measurement of appetite hormones. If consent is given, additional samples will be obtained, these will be stored as plasma samples.

Blood samples for the majority of appetite hormones (if the participant has consented for these samples to take place) will be collected into pre-cooled blood collection tube pre-treated with 250ul of aprotinin (EDTA 4.9ml, 1 tube at each time point) at the same time points that blood sampling for insulin and glucose takes place. Moreover, at the same time points for blood collection for rest of appetite hormones, 2ml of blood will be collected with syringe and will be processed in accordance with the Leicester Diabetes Centre standard operating procedures for blood collection of samples for measurement of acylated ghrelin, a hunger hormone.

After each blood withdrawal the cannula will be flushed with saline to maintain patency. Blood samples will be centrifuged at the point of collection 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.

## 7.8 Randomisation

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 at Visit 1 in a 1:1 ratio to the following treatment sequence:

Participants will be individually randomised (1:1) using a block design to one of the two trial sequences (below);

1) Prolonged sitting for ≈6h 45min (control ) followed by sitting [total ≈6h 15min] and one bout of AEX for 30min

or

2) Sitting (total ≈6h 15min) and one bout of AEX for 30 min followed by prolonged sitting for ≈6h 45min (control).

Randomisation outcome will be revealed by a member of the study team. The independent researchers revealing randomisation allocation will sign the delegation of authority log and follow a local SOP to reveal the randomisation as requested.

Participants will be informed of their randomisation assignment during the Visit 1.

### 7.9 Subsequent Assessments

After completion of visit 0 participants will be contacted and booked to attend visits 1 and 2 and visits 3 to 5 (Visit 1 at least 8 days post the screening visit [visit 0]). Randomisation will take place at Visit 1. After completion of the screening visit (Visit 0) the interventions (Visit 1 to 2 and Visits 3 to 5) will be undertaken over three consecutive days with a three day minimum washout period between them (visit 2 to 3) (maximum washout period will be 28 days). Ideally we would like a washout period to be 3-14 days in duration, however we allow a window of up to 28 days for the washout period (to allow flexibility, i.e. should participant require to self-isolate or household-isolate, etc.).

The outcome measures being collected at each visit are outlined in section 5.2 and listed in table 1. Any adverse events and changes from the screening/ baseline visit (e.g. medications) will be recorded. Due to the short nature of the trial, we anticipate few health related changes to be reported. At the start of the study, participants will be provided with a telephone number to contact the research team with any concerns.

### 7.10 Definition of End of Trial

The end of the trial is defined as when the primary and secondary outcomes are all processed and analysed.

### 7.11 Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time. In addition, the investigator (an individual/ individuals approved by the CI as listed on the delegation of authority log) may withdraw 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/unknown at screening)
- An adverse event which requires discontinuation of the study or results in inability to continue to comply with study procedures
- Disease progression which requires discontinuation of the study or results in inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up
- Subject deceased
- Loss of capacity
- Pregnancy
- Significant protocol deviation according to the judgement of the principal investigator
- Less than 16h 48min CGM data available for analysis over the 24-h period after intervention.

The reason for withdrawal will be recorded in the CRF and medical notes. If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised. Use of identifiable data recorded or tissue collected up until the study withdrawal date will be used in analysis.

### 7.12 Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF and questionnaires). 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). In this study the CRF and questionnaires will be used as

the source documents for the study. All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent form, the participant will be referred to by the study participant number/code, not by name.

## 8. MANAGEMENT OF TRIAL PARTICIPANTS

### 8.1 Description of Study Intervention

The description of interventions is outlined in section 5.

### 8.2 Storage of Study Equipment or Related apparatus

The equipment and standardised meals will be stored in accordance with the Leicester Diabetes Center departmental standard operating procedures.

### 8.3 Compliance with Study Treatment

Attendance throughout the study visits will be recorded and three diaries (3-day diet diary, glucose monitoring diary and wake and sleep log) will be provided along with objective monitoring methods to ensure compliance with suggested food intake and physical activity during the treatment periods. If significant non-compliance with protocol (according to the judgement of the PI) or significant amount of missing data for the primary outcome (for example less than 16h 48min CGM data available for analysis over the 24-h period after intervention), these participants may be excluded from part of the analysis or the whole analysis.

### 8.4 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

### 9.1.1 Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participants, which does not necessarily have to have a causal relationship with this treatment.

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

### 9.1.2 Adverse Reaction (AR)

All untoward and unintended responses related to the study.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study qualify as adverse reactions.

### 9.1.3 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.

### 9.1.4 Serious Adverse Event or Serious Adverse Reaction

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.

### 9.1.5 Expected Serious Adverse Events/Reactions

No serious adverse events/reactions are expected in this study. However, any Serious Adverse Events/Reactions other than death will only be reported to the Sponsor if it is deemed to be related to the study procedures. For example planned routine and elective surgery will not be reported to the sponsor.

### 9.1.6 Suspected Unexpected Serious Adverse Reactions

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

## 9.2 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, will be recorded on the CRF.

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

AEs considered related to the study 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.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment (see section 7.7). 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 severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

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

### 9.3 Reporting Procedures for Serious Adverse Events

All SAEs, except those expected ones defined in section 9.1.5 that do not require immediate reporting (see 9.1.5), must be reported to the Sponsor within one working day of discovery or notification of the event. 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 using the appropriate reporting form and the contact details on there. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form which must be sent to the Sponsor using the appropriate reporting form and the contact details on there.

The Sponsor will report all SUSARs to the Research Ethics Committee concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The CI 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 CI shall submit once a year throughout the study or on request an Annual Report to the Ethics Committee which lists all SAEs / SUSARs that have occurred during the preceding 12 months.

### 9.4 Definitions of hypoglycaemia (HYPO)

A HYPO episode will be defined as treatment emergent if the onset of the episode is on or after the first day of randomised treatment and no later than 24h after completion of the second intervention

in the study. Hypoglycaemic events will be defined as nocturnal if the time onset is between 00:01 and 05:59 (both included).

A HYPO episode form and an Adverse Event (AE) form must be filled in for all HYPO episodes. HYPO episodes requiring the assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions (i.e., 'severe HYPO episodes') will be qualified as an event of special interest and must be reported as an SAE. These will be checked for at each of the visits the participant attends as well as the safety calls conducted. Details of the episode(s) and resolution(s) will be documented.

**Definition of hypoglycaemia during daily life**

All participants will be supplied with a glucose monitoring diary for recording symptoms suggestive of hypoglycaemia and a glucose meter for measurement and documentation of capillary blood glucose levels at the time of the HYPO symptoms, as well as documentation of treatment received. Capillary blood glucose will be measured in all participants when there is a suspicion of HYPO.

All blood glucose values <3.0mmol/L (55mg/dL), as well as values ≥3.0mmol/L (55mg/dL) when hypoglycaemic symptoms have occurred should be recorded by the participants in the diaries. Hypoglycaemic episodes must be transcribed into the CRF by the study team throughout the trial.

**Hypoglycaemia classification during daily life**

Severe hypoglycaemia: An episode requiring assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions. This would be classed as a SAE.

Documented symptomatic hypoglycaemia: An episode during which typical symptoms of hypoglycaemia are accompanied by a measured blood glucose concentration <3.0mmol/L.

Asymptomatic hypoglycaemia: An episode not accompanied by typical symptoms of hypoglycaemia, but with a measured blood glucose concentration <3.0mmol/L.

Probable symptomatic hypoglycaemia: An episode during which symptoms of hypoglycaemia are not accompanied by a blood glucose determination (but that was presumably caused by a plasma glucose concentration <3.0mmol/L).

Relative hypoglycaemia: An episode during which the participant reports any of the typical symptoms of hypoglycaemia and interprets those as indicative of hypoglycaemia, but with a measured blood glucose concentration ≥3.0mmol/L.

**Definition of hypoglycaemia during analysis of the blinded CGM (Dexcom G6)**

An episode of hypoglycaemia on CGM will be defined as at least 2 sensor values <3.0mmol/L (54mg/dl) that were 15 or more minutes apart with no intervening values ≥3.0mmol/l. At least 2 sensor values ≥3.9mmol/l that were 15 or more minutes apart with no intervening values <3.9mmol/l will be required to define the end a hypoglycemic event.

Symptomatic hypoglycaemia during CGM will be defined as an episode of hypoglycaemia on CGM (defined as above) accompanied with typical symptoms of hypoglycaemia which have been documented at the glucose monitoring diary

Asymptomatic hypoglycaemia during CGM will be defined as an episode of hypoglycaemia on CGM (defined as above) not accompanied with typical symptoms of hypoglycaemia or without documentation of symptoms at that time at the glucose monitoring diary.

Probable symptomatic hypoglycaemia: An episode during which symptoms of hypoglycaemia are not accompanied by a interstitial glucose determination (but that was presumably caused by a plasma glucose concentration <3.0mmol/L).

Relative hypoglycaemia: An episode during which the participant reports any of the typical symptoms of hypoglycaemia and interprets those as indicative of hypoglycaemia, but with interstitial glucose levels ≥3.0 mmol/L.

**Definition of hypoglycaemia during the mixed meal tolerance test (MMTT)**

Asymptomatic hypoglycaemia during the MMTT will be defined as plasma glucose <3.0 mmol/l not accompanied with typical symptoms of hypoglycaemia.

Symptomatic hypoglycaemia during the MMTT will be defined as plasma glucose <3.0 mmol/l accompanied with typical symptoms of hypoglycaemia.

Relative hypoglycaemia during MMTT will be defined as an episode during which the participant reports any of the typical symptoms of hypoglycaemia and interprets, but plasma glucose levels are ≥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 the participant experiences symptoms suggestive of hypoglycaemia and venous blood glucose/capillary blood glucose levels are <3.0mmol/l, then the participant will receive treatment for HYPO in accordance with HYPO Guideline for Adults of the UHL and the blood collection for measurement of insulin, glucose and appetite biomarkers as well as the VAS score collection for hunger and satiety during the MMTT will be terminated. The participant will remain in sitting position after HYPO treatment until the expected time of completion of the experimental period. 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 bariatric surgery.

## 10. RESPONSE TO THE COVID-19 GLOBAL PANDEMIC

Due to the current global pandemic we will not encourage participants to attend or take part if it poses a risk to them. We will encourage participants to attend visits by themselves to reduce people coming into the Leicester Diabetes Centre. Prior to each visit participants will also undergo a COVID-19 check which includes a temperature check and some questions and if participants are able to they must wear a face mask/covering when they attend their appointment as per NHS Trust Guidance.

The risk of COVID-19 infection will be minimised by staff wearing PPE and following NHS Trust Guidance and having Study Risk Assessments in place to reduce risk of infection.

To ensure safety of participants and staff at Leicester Diabetes Centre, all clinical areas used for research visits will have Risk Assessments completed to ensure mitigations are in place where risks are identified - this will be carried out prior to starting any study visits. Study based Risk Assessments will be completed also 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 delivery in a safe manner. Visits 0, 1, 2 and 4 will take place as face to face visits due to the nature measurements and procedures required for this study. Visits 3 and 5 can be optional to attend where CGM device would not require re-insertion or to be removed by study team.

If the study team is required to visit GP Practice, community venues or any other organisation, they must adhere to COVID-19 National Government Guidance and any other measures in place within the individual organisation.

# 11. STATISTICS

## 11.1 Description of Statistical Methods

A CONSORT study flow diagram will detail the movement of participants throughout the BariEX study. Baseline descriptive characteristics will be summarised, with continuous variables expressed as mean values (and standard deviations) or median values (with lower and upper quartiles) where appropriate, and binary and categorical variables expressed as number (percentage). Where CGM data is missing from a given trial, the corresponding data will be excluded from the other trial during the same time period at the second treatment. Participants with less than 16-hours 48min CGM data available for analysis over the 24-hour period after the intervention will be excluded from the complete cases analysis. The primary outcome of the study will be the difference in the time spent in HYPO (defined as glucose levels <3.0 mmol/l as measured by CGM) between the AEX and control intervention during the first 24-h post-intervention.

For outcomes related to the standardized MMTT (secondary outcomes), data from blood samples collected pre-MMTT at lunchtime (exactly before MMTT consumption) and 15, 30, 60, 90, 120, 150 and 180 minutes after MMTT consumption (last mouthful of MMTT) will be used to calculate time-averaged AUC (Area Under the Curve), using the trapezium rule. In instances of symptomatic hypoglycaemia during the MMTT(where blood collection for glucose, insulin and appetite biomarkers as well as VAS scores for satiety and hunger will be terminated early), these participants will be excluded from the analysis of secondary outcomes related to the MMTT. Missing data on insulin, glucose, appetite biomarkers and VAS scores for satiety and hunger *within* a completed MMTT will be imputed using a linear regression method as previously reported (35, 36), provided baseline/pre meal value and ≥50% of data points within the MMTT are available. The primary and secondary outcomes will be analysed using GEE (Generalised Estimating Equations) with an exchangeable correlation matrix, taking into account repeated measures across conditions. Outcome distribution will be assessed, with Gamma models used for positively skewed data. Identity and log link functions will be explored with Gamma models, with an identity link preferred (to aid interpretation of results) unless log link provides a substantially superior model fit.

The primary and main secondary outcomes will be analysed using a complete case approach. A sensitivity analysis will also be conducted where individuals in whom rescue action for symptomatic hypoglycaemia during the first 24-h post-intervention was required will be excluded from the

analysis. A second sensitivity analysis will be performed using individuals with any CGM data (i.e. including those with CGM data available for analysis is <16hours 48mins for the first 24 hours post-intervention). A value of P<0.05 will be considered statistically significant for all analyses. Statistical analyses will be carried out using commercially available statistical programmes.

### 11.2 The Number of Participants

This is a proof-of-concept study and there are no previous data available on the acute effect of AEX on time spent on HYPO after bariatric surgery. Previous studies have found that people without diabetes after bariatric surgery spend on average 71±25 min/day in HYPO postoperatively (37) and that the incidence may be similar between RYGB and SG (13). Based on this previous research, the current we estimate that the research project is powered (80%) to detect a 25% difference (18 minutes/day) in time spent in HYPO during the first 24-hs between the AEX and control interventions, with 2-sided alpha set at 5% and assuming a standard deviation of 25min and a moderate within-person correlation of 0.6. On this basis, 18 participants are required for this crossover study (accounting for an estimated drop-out of 20%).

### 11.3 The Level of Statistical Significance

The results of all comparative analyses will be presented with 95% confidence intervals and statistical significance for main effects will be assessed at the 5% level. All p-values shown will be two-sided.

### 11.4 Criteria for the Termination of the Trial.

There are no specific criteria for terminating the trial prematurely. The end of the trial is defined as when primary and secondary outcomes are all analysed.

### 11.5 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any deviation(s) from the original statistical analysis plan will be described and justified in the final report.

## 12. 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 trial-related monitoring, audits and inspections.

## 13. 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 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.

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.

## 14. CODES OF PRACTICE AND REGULATIONS

### 14.1 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 participants at the screening part of Visit 0 and subsequently before each procedure is performed.

The overall care and comfort of the participant will always be considered paramount during the study. The randomised crossover study design ensures random allocation to intervention sequence. The inclusion and exclusion criteria ensures patients are not included if there is a risk of damage to their health through exercise.

### 14.2 Standard Operating Procedures

All relevant Sponsor, host organisation and study specific SOPs will be followed where applicable to ensure that this study complies with all relevant legislation and Guidelines.

### 14.3 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).

### 14.4 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.

### 14.5 Approvals

Once Sponsor authorisation has been confirmed, all study documentation will be submitted to appropriate regulatory bodies (e.g. REC/ HRA), the sponsor and the host institution for written approval. The study will commence upon receiving Sponsor green light.

Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all amendments.

### 14.6 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 by 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 study, 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.

## 15. 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 Hospitals 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 Hospitals 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 Hospitals of Leicester NHS Trust). Final data analysis will be conducted on servers owned and maintained by the host NHS organisation (University Hospitals 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.

Eleven glucose blood samples (11 per visit, 22 in total) per patient will be sent for analysis in UHL laboratories following visits 2 and 4, these results will be sent to the research team to be entered in the CRF. The 11 glucose samples taken for the study will be destroyed once analysed in accordance with the UHL's SOP's. Further to this, insulin and biochemical markers of appetite will be stored as

plasma samples and will be kept until analysed, if consent is obtained (for the biochemical markers of appetite) and if feasible (for the biochemical markers of appetite).

If participants consent for their samples to be stored for future research then plasma will be collected into monovettes for analysis. Blood samples will be centrifuged at the point of collection with the plasma stored in microtubes in a -80 degree freezer. As these bloods are not being stored as whole blood, they do not require HTA licensed laboratory storage. These plasma samples will be stored in a research laboratory at Leicester General Hospital for future ethically approved research. We are unable to specify a period of time for storage of the plasma samples for measurement of appetite biomarkers.

## 16. STUDY GOVERNANCE

### 16.1 Trial Management Group

A Trial Management Group will be set up, to ensure that the study is conducted in accordance with the principles of good clinical practice. The Trial Management Group will include the chief investigator, other senior investigators and the day-to-day project management team. The group will concentrate on the progress of the trial, adherence to the protocol, participant safety, and the consideration of new information that might be of relevance to the research question.

### 16.2 Trial Steering Committee

A trial steering committee is not required for the current study.

### 16.3 Data Safety Monitoring Committee

A data monitoring committee is not required for the current study.

## 17. PUBLICATION POLICY

The trial will be registered on a publicly accessible database prior to study initiation. The findings of the research will be presented at local and national conferences and will be submitted for publication in relevant peer-reviewed journals. The Leicester Diabetes Centre's publication policy will be followed.

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**FIGURE 1: Study Flow Chart**

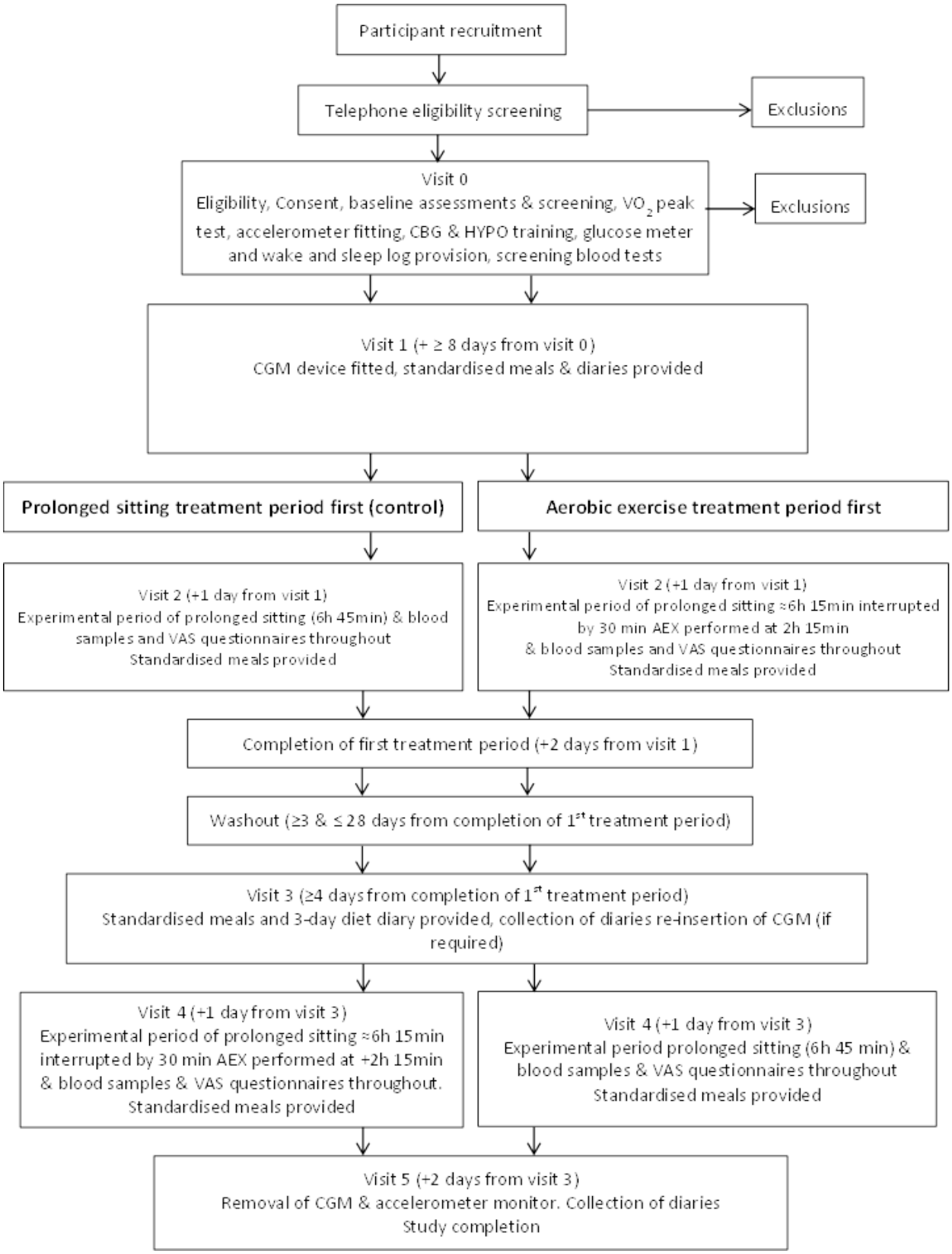
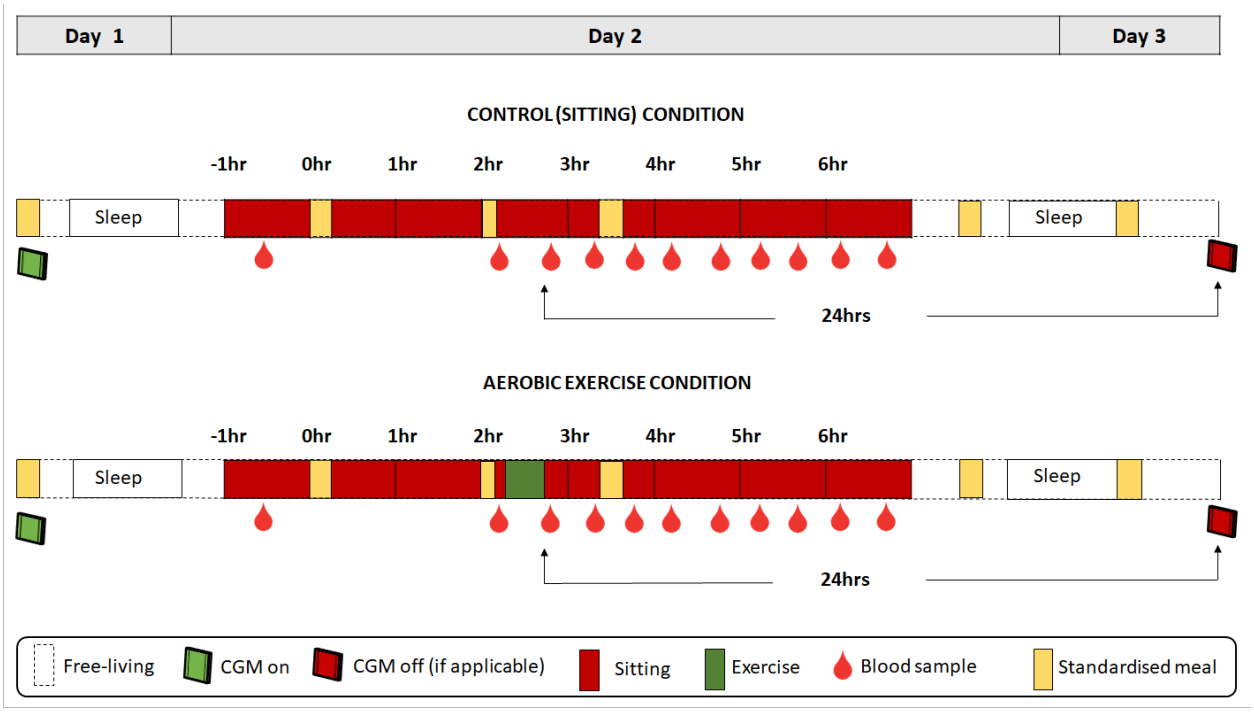

Participant recruitment
Telephone eligibility screening
Exclusions
Visit 0
Eligibility, Consent, baseline assessments & screening, VO2 peak test, accelerometer fitting, CBG & HYPO training, glucose meter and wake and sleep log provision, screening blood tests
Exclusions
Visit 1 (+ ≥ 8 days from visit 0)
CGM device fitted, standardised meals & diaries provided
Prolonged sitting treatment period first (control)
Aerobic exercise treatment period first
Visit 2 (+1 day from visit 1)
Experimental period of prolonged sitting (6h 45min) & blood samples and VAS questionnaires throughout
Standardised meals provided
Visit 2 (+1 day from visit 1)
Experimental period of prolonged sitting ≈6h 15min interrupted by 30 min AEX performed at 2h 15min
& blood samples and VAS questionnaires throughout
Standardised meals provided
Completion of first treatment period (+2 days from visit 1)
Washout (≥3 & ≤ 28 days from completion of 1st treatment period)
Visit 3 (≥4 days from completion of 1st treatment period)
Standardised meals and 3-day diet diary provided, collection of diaries re-insertion of CGM (if required)
Visit 4 (+1 day from visit 3)
Experimental period of prolonged sitting ≈6h 15min interrupted by 30 min AEX performed at +2h 15min
& blood samples & VAS questionnaires throughout.
Standardised meals provided
Visit 4 (+1 day from visit 3)
Experimental period prolonged sitting (6h 45 min) & blood samples & VAS questionnaires throughout
Standardised meals provided
Visit 5 (+2 days from visit 3)
Removal of CGM & accelerometer monitor. Collection of diaries
Study completion

**FIGURE 2 – Blood sampling process**

This is an example of the sitting condition followed by the exercise condition, 50% of participants will perform this the other way around (the exercise condition followed by the sitting condition).



## Appendix 1 – Visual analogue scale

# Visual Analogue Scale

**Time:**

**Place a mark on the horizontal lines below after considering the following questions:**

**How hungry do you feel?**

I am not hungry at all — I have never been more hungry

**How satisfied do you feel?**

I am completely empty — I cannot eat another bite

**How full do you feel?**

Not at all full — Totally full

**How much do you think you can eat?**

Nothing at all — A lot

**How nauseous do you feel?**

Not at all nauseous — Very nauseous

| Temperature (°C) | Humidity (%) |
|---|---|
| | |

## Visual Analogue Scale

**Time:**

**How pleasant was this meal?**

Not at all pleasant ——————— Extremely pleasant

**How satisfying was this meal?**

Not at all satisfying ——————— Extremely satisfying

**Appendix 2 - International Physical Activity Questionnaire Short Form (IPAQ-SF)**

# INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

**Appendix 3 – Table 2. Study procedures during the study.**

| **Visits (No)** | Telephone screening | Baseline Visit 0 (2.5 h) | Visit 1 (30min) | Visit 2 (6.45h) | Washout period (≥3&≤28days) **Ideally ≥3&≤14** | Visit 3 (20min) (≥3&≤29 days) **Ideally** | Visit 4 (6.45min) | Visit 5 (20min) |
|---|---|---|---|---|---|---|---|---|
| **Key time points** | Upon return of EOI | MIN 24h before visit 1 | + ≥8 & ≤12 days from visit 0 | +1 day from visit 1 | +≥3 & ≤28 days from completion of 1st treatment period | +≥4&≤29 days from completion of 1st treatment | +1 day from visit 3 | +2 days from visit 3 |
| **Study procedures** | | | | | | | | |
| Non-measured screening criteria (Telephone) | x | | | | | | | |
| Initial consent to come for visit 0 | x | | | | | | | |
| Inclusion/exclusion criteria | | x | X# | X# | | X# | X# | X# |
| Informed consent | | x | x | x | | x | x | x |
| Screening measurements | | x | | | | | | |
| Demographic data | | x | | | | | | |
| Medical/Surgical history | | x | | | | | | |
| Prescribed and concomitant | | x | | | | | | |
| Change in concomitant medication and diseases | | x | x | x | | x | x | x |

| **Visits (No)** | Telephone screening | Baseline Visit 0 (2.5 h) | Visit 1 (30min) | Visit 2 (6.45h) | Washout period (≥3&≤28days) **Ideally ≥3&≤14** | Visit 3 (20min) (≥3&≤29 days) **Ideally** | Visit 4 (6.45min) | Visit 5 (20min) |
|---|---|---|---|---|---|---|---|---|
| **Key time points** | Upon return of EOI | MIN 24h before visit 1 | + ≥8 & ≤12 days from visit 0 | +1 day from visit 1 | +≥3 & ≤28 days from completion of 1st treatment period | +≥4&≤29 days from completion of 1st treatment | +1 day from visit 3 | +2 days from visit 3 |
| Filling CRF forms | | x | x | x | | x | x | x |
| Database | | x | x | x | x | x | x | x |
| Anthropometric | | x | | x | | | x | |
| Height | | x | | | | | | |
| Body Mass | | x | | x | | | x | |
| Body Mass index | | x | | x | | | x | |
| Waist circumference | | x | | x | | | x | |
| Cardiovascular measures | | x | | x | | | x | |
| Systolic and diastolic blood pressure | | x | | x | | | x | |
| Resting heart rate | | x | | x | | | x | |
| Accelerometer fitting | | x | | | | X~ | | |
| Accelerometer removal | | | | | | | | x |
| Mifflin calculation | | x | | | | | | |
| Maximal exercise test and ECG | | x | | x | | x | | |

| **Visits (No)** | Telephone screening | Baseline Visit 0 (2.5 h) | Visit 1 (30min) | Visit 2 (6.45h) | Washout period (≥3&≤28days) **Ideally ≥3&≤14** | Visit 3 (20min) (≥3&≤29 days) **Ideally ≥4&≤15** | Visit 4 (6.45min) | Visit 5 (20min) |
|---|---|---|---|---|---|---|---|---|
| **Key time points** | Upon return of EOI | MIN 24h before visit 1 | + ≥8 & ≤12 days from visit 0 | +1 day from visit 1 | +≥3 & ≤28 days from completion of 1st treatment period | +≥4&≤29 days from completion of 1st treatment | +1 day from visit 3 | +2 days from visit 3 |
| Glucose meter and strips provision | | x | | | | | | |
| Glucose meter | | | | | | | | x |
| CBG training | | x | X* | | | X* | | |
| Randomisation | | | x | | | | | |
| CGM device fitted | | | x | | | Xa | | |
| CGM device worn (Continuous glucose monitoring) | | | x | x | x | x | x | x |
| CGM device removal | | | | | | | | x |
| Standardised meals provision | | | x | x | | Xc | x | |
| Snack provision | | | | x | | | x | |
| 30 min of AEX | | | | Xd | | | Xd | |
| Mixed meal tolerance test (MMTT) | | | | x | | | x | |
| Cannula insertion | | | | x | | | x | |
| Blood sampling | | X(1) | | X(11) | | | X(11) | |
| Blood glucose monitoring | | | | X(11) | | | X(11) | |

| **Visits (No)** | Telephone screening | Baseline Visit 0 (2.5 h) | Visit 1 (30min) | Visit 2 (6.45h) | Washout period (≥3&≤28days) **Ideally ≥3&≤14** | Visit 3 (20min) (≥3&≤29 days) **Ideally** | Visit 4 (6.45min) | Visit 5 (20min) |
|---|---|---|---|---|---|---|---|---|
| **Key time points** | Upon return of EOI | MIN 24h before visit 1 | + ≥8 & ≤12 days from visit 0 | +1 day from visit 1 | +≥3 & ≤28 days from completion of 1st treatment period | +≥4&≤29 days from completion of 1st treatment period | +1 day from visit 3 | +2 days from visit 3 |
| Centrifuge of samples | | | | X(11) | | | X(11) | |
| Storing of samples | | | | X(11) | | | X(11) | |
| Urine Pregnancy test for all female patients of childbearing potential | | x | | | | | | |
| **Questionnaires and diaries** | | | | | | | | |
| VAS issued, completion and collected | | | | X(11) | | | X(11) | |
| Blood glucose monitoring diary provided | | | x | | | | | |
| Glucose monitoring diary completion | | | x | x | x | x | x | x |
| Glucose monitoring diary collection | | | | | | x | | x |
| 3-day diet diary issued | | | x | | | x | | |
| 3-day diet diary completion | | | x | x | | x | x | x |
| 3-day diet diary collection | | | | | | x | | x |
| Wake and sleep log issued (activity | | x | | | | | | |
| Wake and sleep log completion | | | x | x | X(optional) | x | x | x |

| **Visits (No)** | Telephone screening | Baseline Visit 0 (2.5 h) | Visit 1 (30min) | Visit 2 (6.45h) | Washout period (≥3&≤28days) **Ideally ≥3&≤14** | Visit 3 (20min) (≥3&≤29 days) **Ideally** | Visit 4 (6.45min) | Visit 5 (20min) |
|---|---|---|---|---|---|---|---|---|
| **Key time points** | Upon return of EOI | MIN 24h before visit 1 | + ≥8 & ≤12 days from visit 0 | +1 day from visit 1 | +≥3 & ≤28 days from completion of 1st treatment period | +≥4&≤29 days from completion of 1st treatment | +1 day from visit 3 | +2 days from visit 3 |
| Wake and sleep log collection | | | | | | x | | x |
| IPAQ-SF issued and collected | | x | | | | | | |

X#: Check for any changes in exclusion criteria (e.g. course of steroids)

X~: Should accelerometer battery run out, then a second accelerometer will be issued for the second treatment period.

X* Education reminders

Xa: Re-insertion of CGM (if required). If the period between visit 1 and visit 5 is more than 10 days (which is the recommended use for the Dexcom G6 sensor), then a second CGM sensor (Dexcom G6) will be inserted at visit 3 for the second treatment period.

Xb If there are issues regarding sensor life (for example if the end of the second treatment period is more than 10 days from Dexcom G6 sensor insertion), a second CGM sensor will be issued at visit 3 where necessary, together with a new glucose diary.

Xc In a scenario of visit 3 being carried out via phone or video call meals will be provided as required in advance of this visit

Xd depending on sequence, on either visit