Full Study Title: Effects of serotonin receptor agonism on blood glucose lowering: Proof of concept in humans

Short title: Sumatriptan and glucose

IRAS Ref: 277675

Date and Version No: 01/11/2020, Version 2.0

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Sponsor:	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge

AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2	1Nov 2020	Dr Rajna Golubic	Changes were made to address the REC comments. Inclusion criteria have been changed to include participants who are overweight rather than obese due to the risk of COVID19 as recommended by the Scientific Advisory Board of the Clinical Research Facility (Cambridge). Timeline has been amended to account for the effects of pandemic.

List details of all protocol amendments here whenever a new version of the protocol is produced.

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:

Name (please print): Jacob Wingfield

Position: Research Governance Coordinator (Division D)

Chief Investigator:

Signature: Date: 08/11/2020

Name (please print): Mark Evans

KEY STUDY CONTACTS

Insert full details of the key study contacts including the following

Chief Investigator	Full contact details including phone, email and fax numbers Dr Mark Evans, email: mle24@cam.ac.uk, phone: 01223 748471 Principal investigator: Dr Rajna Golubic, email: rg380@medschl.cam.ac.uk; mob: 07411730033
Study Co-ordinator	Full contact details including phone, email and fax numbers Jane Kennet, email: jk605@cam.ac.uk; phone: 01223 748471
Sponsor	Full contact details including phone, email and fax numbers The sponsor can be defined as the individual, company, institution, or organisation assuming overall responsibility for the initiation and management of the study, and is not necessarily the main funder. Sponsorship responsibilities may be shared by joint- or co-sponsors Cambridge University Hospitals NHS Foundation Trust Stephen Kelleher Research & Development Department Cambridge University Hospitals NHS Foundation Trust |Box 277 | Addenbrooke's Hospital | Hills Road |Cambridge | CB2 0QQ Tel: 01223 256620 (internal ext 256620) Email: research@addenbrookes.nhs.uk
Joint-sponsor(s)/co-sponsor(s)	Full contact details including phone, email and fax numbers of ALL organisations assuming sponsorship responsibilities as a joint- or co-sponsor/s (If applicable) University of Cambridge Stephen Kelleher, research@addenbrookes.nhs.uk NB Same contact person for both the Cambridge University Hospitals NHS Foundation Trust and the University
Funder(s)	Names and contact details of ALL organisations providing funding and/or support in kind for this study Diabetes Research and Wellness Fund Sarah Brown, research administrator Email: sarah.brown@drwf.org.uk; Phone: +44 23 9263 6135

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SYNOPSIS

It may be useful to include a synopsis of the study for quick reference. Delete or alter as appropriate/required.

Study Title	Effects of serotonin receptor agonism on blood glucose lowering: Proof of concept in humans (Acronym: Sumatriptan and glucose)
Internal ref. no.	A095470
Study Design	Cross-over randomised trial
Study Participants	Overweight adults without known health co-morbidities
Planned Sample Size (if applicable)	10 to 12
Planned Study Period	6 months
Primary Objective	To establish proof of concept and examine mechanisms for $5HT_{1B}$ agonism to affect measures of blood glucose homeostasis in humans. Specifically, to assess whether a single dose of sumatriptan (tablet) improves insulin sensitivity and/or insulin secretion.

STUDY FLOW CHART

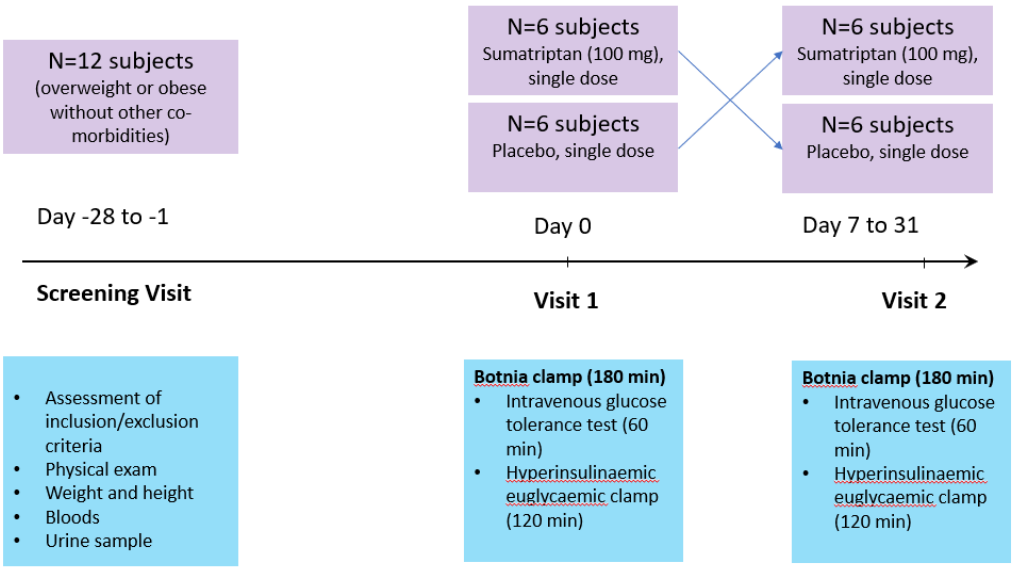

Figure 1 Flow diagram of the study

NB Timeline of the study is demonstrated in Appendix 2.

1. BACKGROUND AND RATIONALE

Background and justification for the research

In healthy individuals, blood glucose levels are tightly controlled within a narrow range despite various metabolic perturbations. Glucose homeostasis is achieved by rapid changes in insulin release from the pancreas, insulin action at level of liver, muscle and fat, and non-insulin dependent actions. In addition to these effects, increasing data suggest that brain may play an important central role, detecting changes in glucose and integrating the actions of peripheral organs to effect glucose homeostasis (1-4). Where these homeostatic processes fail, changes in blood glucose levels can result in severe clinical consequences in short and long term.

Serotonin (5HT) is a neurotransmitter which is involved in a number of important functions including energy balance (promoting weight loss), mood control and reproduction, acting via a family of receptors. Data from mouse studies which we participated in suggest that serotonin in brain exerts a blood glucose lowering effect by signalling through two of these receptors ($5HT_{2C}$ and $5HT_{1B}$ receptors).

In this study, we aim to examine whether activation of brain serotonin receptors can alter blood glucose homeostasis in humans. The scientific rationale and evidence from research including animal models are outlined below.

$5HT_{2C}$ receptors

Using mouse models, we examined the effects of lorcaserin on glucose homeostasis and the central pathways involved (1). In summary, lorcaserin improved glucose handling in mice in a dose-dependent fashion independent of effects on weight loss. Employing targeted genetic mouse models, we found that lorcaserin improved glucose metabolism by acting through the brain's melanocortin pathway projecting to brainstem cholinergic neurones. In these studies, the net effect was to increase the sensitivity of the liver to insulin, but we also saw a trend towards improved insulin secretion and glucose effectiveness.

Until February 2020, the only commercially available $5HT_{2C}$ receptor agonist was lorcaserin (5) which was exclusively licensed globally to Eisai (Tokyo) from 2017 until February 2020. A large global cardiovascular outcome trial performed in overweight people with and without diabetes (CAMELLIA-TIMI61, N=12,000, 57% diabetes, 33% pre-diabetes) reported no obvious safety concerns with lorcaserin, including echocardiographic surveillance in a large subset (6). This was a particular focus as previous less selective 5HT-receptor agonists

(e.g."phen-fen") were associated with cardiac valvulopathy, probably by broad actions of serotonin released by fentermine on cardiac $5HT_{2B}$ receptors (7). The CAMELLIA-TIMI61 study also showed improvements in measures of blood glucose control in those on lorcaserin (reduced HbA1c, reduced rates of new-onset diabetes) (6). Because lorcaserin resulted in weight loss, it is unclear from this study whether these improvements in glycaemia are explained by the modest weight loss seen or whether there were also direct effects on blood glucose.

We had initially planned therefore to examine the effects of lorcaserin on glucose homeostasis in humans and indeed the original funding award from the Diabetes Research and Wellness Foundation (DRWF) was to support testing of this hypothesis. However, shortly after the award was confirmed, an analysis of 5y follow-up data of the CAMELLIA-TIMI61 trial conducted by Eisai (Tokyo) reported that N=462 (7.7%) patients taking lorcaserin developed cancer compared to N=423 (7.1%) in the placebo group. Although this possible cancer signal appears to be small, the US Food & Drug Administration (FDA) deemed lorcaserin to be unsafe and withdrew it from the market (8). We felt it inappropriate to proceed with the study as planned and there are no alternative available $5HT_{2C}$ receptor agonists suitable for human dosing. After discussion with funders and with approval from the DRWF, we switched our focus to examine our alternative candidate $5HT_{1B}$ pathway as described below.

$5HT_{1B}$ receptors

Following the lorcaserin mouse research, we have continued to collaborate in preclinical studies with Professor Lora Heisler's team at the University of Aberdeen. Her team have generated strong evidence from mouse models using sumatriptan ($5HT_{1B}$ receptor agonist) to improve glucose homeostasis suggesting that $5HT_{1B}$ receptor acts synergistically with $5HT_{2C}$ receptor signalling in this central pathway controlling glucose (personal communication with Prof Heisler). Much like the $5HT_{2C}$ receptor, the synergism was first identified for appetite/energy (9) but Prof Heisler's current unpublished work shows that this extends to glycaemic control. We aim therefore to examine effects of sumatriptan on glucose homeostasis in overweight individuals without known diabetes or other medical conditions. The rationale for selecting this participant group is that effects to improve glucose homeostasis were seen with insulin resistance (obese rodent) models. Sumatriptan is currently used for the treatment of migraine worldwide and its side effect profile has been well characterised (10). We are currently unaware of any other ongoing similar studies examining glycaemic actions of sumatriptan either from personal communication and searching ClinicalTrials.gov (11).

The ultimate rationale behind this study is that this may allow future therapies to be developed for managing diabetes (either repurposing of sumatriptan and/or establishment of a new clinical niche for other $5HT_{1B}$ agonists).

Main research questions and aims

The purpose of this study is to establish the proof of concept for a brain serotonin pathway controlling blood glucose control in humans. We will examine whether activation of serotonin receptors ($5HT_{1B}$) by a single dose of sumatriptan (a drug used for the treatment of migraine worldwide) can lead to short term changes in blood glucose homeostasis. Thus far, this has been shown only in animal models but no study in humans has directly investigated this research question.

Briefly, this study will be performed with a single dose of sumatriptan (100 mg) in overweight, otherwise healthy humans. If sumatriptan alters glucose control, this might support future testing in disease models i.e. people with type 1 diabetes (T1D) and/or type 2 diabetes (T2D). Ultimately, if successful, either sumatriptan could be repurposed and/or other $5HT_{1B}$ agonists (triptans) could be developed for diabetes.

Study population and intervention

The study design and procedures are described in detail in the relevant section of this form. Briefly, we will recruit 10-12 overweight volunteers without other known significant co-morbidities who will attend our clinical research facility on 2 occasions for physiological studies conducted as “day cases”. We will use a double blinded placebo controlled cross-over design whereby each participant will be her/his own control and will receive a single dose sumatriptan or placebo which will be followed by a physiological study (“Botnia clamp”) performed over 3h in the Translational Research Facility (affiliated with the Cambridge University Hospital). On a separate occasion at least one week later, those who received sumatriptan will receive placebo and *vice versa* (random order) and the Botnia clamp will be repeated. The Botnia clamp consists of targeted infusions of glucose and insulin with frequent blood sampling as detailed in the Section 4.3.

Risks and benefits of the procedure

The procedure risks include cannulation failure, bleeding, bruising, skin irritation and infection. Well trained staff, standardised protocols and aseptic technique are the strategies used to minimise these risks. Other risks include well established side effects of sumatriptan (Appendix 4) which are explained in the Patient Information Sheet.

OBJECTIVES

2.1 Primary Objective

To establish proof of concept and examine mechanisms for $5HT_{1B}$ agonism to improve measures of blood glucose homeostasis in humans.

Specifically, the primary objective is to assess (in healthy humans) whether a single dose (100 mg) of sumatriptan can:

1) alter insulin sensitivity

2) alter glucose-stimulated insulin secretion.

2.3 Outcome

The following outcomes are expected:

1) Increased insulin sensitivity with sumatriptan compared to placebo (measured here as an "M-value" which is defined as average glucose infusion rate when euglycaemic steady state has been reached after the start of insulin infusion, and is expressed in milligrams of glucose per kilogram of body weight per minute).

2) Increased glucose stimulated insulin secretion with sumatriptan compared to placebo- acute insulin secretory response measured during the intravenous glucose tolerance test (ivGTT) phase of the Botnia clamp. Note, we did not see this in our murine studies underpinning this project but data suggest a trend for improvement (1).

3) Based on the above, we anticipate established proof of concept for further appropriately targeted studies in people with diabetes.

2. STUDY DESIGN AND METHODS

The design is a random order double blinded placebo controlled cross-over design of “day case” physiological studies with at least 1 week between studies. A single dose of sumatriptan (100 mg) or placebo allows inpatient dispensing and eliminates the effects of weight loss as a potential confounder. This proof of concept study involves performing detailed physiological studies in overweight adults (aged 18 to 65 years) without other known medical conditions following ingestion of a single dose of sumatriptan or placebo. The study will take place in the Translational Research Facility (TRF) which is embedded in the NIHR/Wellcome Trust Clinical Research Facility affiliated with the Cambridge University Hospital.

All subjects (10 to 12 volunteers) will undergo a screening visit. This will include an assessment of inclusion/exclusion criteria (outlined below), physical exam, alcohol and substance screening test, pregnancy test for women and routine blood tests (full blood count, urea and electrolytes, HbA1C, liver function tests, blood borne viruses).

We have research infrastructure and personal expertise to perform both insulin clamp studies (12) and frequently sampled intravenous glucose tolerance tests (FSivGTT). We have a research pharmacy who can source and repackage sumatriptan / placebo. The assessment of insulin sensitivity and insulin secretion usually requires separate study days. In this study, we aim to combine these into a single 3 hour physiological study combination (FSivGTT followed by a hyperinsulinaemic euglycaemic clamp (HEC) sometimes termed a “Botnia clamp”) (13). This markedly reduces the burden on participants and is the most parsimonious way for us to deliver this proof of concept study. We will study participants without diabetes to allow us to assess both insulin secretion and sensitivity. The Botnia clamp technique is described in detail in the Study Procedures and Interventions section. Data will be collected at the screening visit and subsequent 2 visits by the principal investigator and research nurses. The principal investigator will analyse data at the end of the study. Microsoft Excel and STATA version 16 (14) will be used for analysis.

3.1 Study Participants

Ten to twelve overweight otherwise healthy volunteers will be studied. All subjects must give their written informed consent to participate in the study which will be performed according to the principles of Good Clinical Practice (GCP) & the Declaration of Helsinki (latest revision). Subjects will be given a Participant Information Sheet with information about the study written in lay language. Eligibility criteria and concomitant medications will be reviewed at the screening visit and both subsequent visits to ensure safety.

3.2 Inclusion Criteria

1. Being able to provide a written informed consent
2. Age between 18 and 65 years
3. Body Mass Index (BMI) ≥25 kg/m^2 and <30 kg/m^2 for non-Asian individuals and BMI≥23 kg/m^2 and <25 kg/m^2 for Asian individuals according to the BMI classification by the World Health Organization (WHO)
4. HbA1C<48 mmol/mol at screening
5. Subject must not use any regular prescribed medications (this excludes simple analgesia used as needed)
6. Subject must not use any over the counter supplements targeting metabolism
7. Subject must not have any acute or chronic disease which in the opinion of the investigator may affect the study outcome
8. Subject must not be a current smoker
9. No history of substance abuse or excess alcohol consumption (>14 units/week)
10. Women of childbearing age must have a negative pregnancy test at screening and must not be breastfeeding
11. Women of childbearing age who are sexually active with a male partner must use highly effective contraceptive methods

3.3 Exclusion Criteria

1. Use of any regular medications
2. Use of illicit drugs
3. Use of any over the counter supplements affecting metabolism
4. Diagnosis of any acute / chronic disease
5. Current smoking or excess alcohol consumption (>14 units/week)
6. Current pregnancy or lactation
7. Abnormal findings on physical exam or routine blood tests at screening (full blood count, urea and electrolytes, HbA1C, liver function tests)
8. Concurrent participation in another trial with an investigational product
9. History of anaphylaxis

4. STUDY PROCEDURES AND INTERVENTIONS

Describe all study procedures and assessments in detail. Add visit numbers as appropriate. Add schedule of procedures as an appendix if appropriate.

4.1 Recruitment

We expect to screen approximately 15 volunteers to allow for screen failures and plan to recruit 10 to 12 volunteersPrior to screening a telephone call will be conducted to broadly assess eligibility criteria and guide the decision if an individual should undergo a screening visit. We aim to use local advertising first (posters in the Wolfson Diabetes and Endocrine Clinic, Cambridge University Hospitals NHS Foundation Trust electronic newsletter) which we have used effectively before. In addition, we have a database of overweight and obese

people who have indicated their willingness to be approached about participating in clinical research. If these strategies yield insufficient number of participants, we will liaise with local GP surgeries which is another recruitment strategy we have successfully used in the past. The study will also be advertised in the social media. Participants will be financially compensated for participation and transport costs (£100).

4.2 Informed Consent

Written informed consent will be obtained at the screening visit (Appendix 1). We have included Participant Information Sheet (PIS) and Informed Consent Form (ICF) as a separate document for a review by the Independent Ethics Committee. The PIS contains key details of the study written in lay language and the flow chart of procedures. The risks and benefits of participation in this study are clearly outlined in the PIS. During this process, the participants will receive this PIS at least 24 hours before the screening visit and will have the opportunity to ask questions and discuss any concerns they may have about the participation in the study. Given that the target population group are overweight healthy volunteers, they will all have mental capacity to independently make the decision about participation. The PIS clearly highlights that participation in the study is voluntary and subjects can withdraw at any point.

The principal investigator will ensure that:

- each participant is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study
-each participant is notified that they are free to discontinue from the study at any time
-each participant is given the opportunity to ask questions and allowed time to consider the information provided
-each participant provides signed and dated informed consent before conducting any procedure specifically for the study
-that the original, signed ICF(s) are stored in the Investigator's Study File
-a copy of the signed ICF is given to each participant
-that any incentives for participants in the study and any provisions for participant harmed as a consequence of study participation are described in the ICF that is approved by an Independent Ethics Committee.

4.3 Study Assessments/Interventions

All subjects will undergo a screening visit. This will include: assessment of inclusion / exclusion criteria; height and weight measurement (using standard calibrated stadiometers and scales, respectively); physical exam; urine sampling for alcohol and substance screening

test; urine pregnancy test for women, and routine blood tests (full blood count, urea and electrolytes, liver function tests and HbA1C). Eligible subject will proceed to Visit 1 (day 0) between 1 to 28 days after the screening visit and Visit 2 (day 7 to 31 after visit 1) at which they will receive a single dose sumatriptan (100mg) or placebo in a random order and Botnia clamp studies (described in detail below) will be performed.

We have research infrastructure and personal expertise to perform these physiological studies (12). In this study, we aim to combine FSivGTT and hyperinsulinaemic euglycaemic clamp (HEC) into a single 3 hour physiological study combination (FSivGTT followed by a HEC sometimes termed a “Botnia clamp”) (13). This technique has been reported to have a high reproducibility and validity compared to traditional HECs in non-diabetic individuals with and without family history of T2D (13). The assessment of insulin secretion and sensitivity typically takes place on separate days. Combining them in a single 3h study substantially reduces the burden on participants and is the most parsimonious way for us to deliver this proof of concept study as alluded to earlier. We will study participants without diabetes to allow us to assess simultaneously both insulin secretion and sensitivity.

Botnia clamp technique

The procedure will be undertaken in the morning following an overnight fast of at least 10h. Participants will remain rested on a bed during the procedures. It lasts 3h and consists of two components: 1) FSivGTT (60 min) followed by a 2) HEC for the remaining 120 min.

1) FSivGTT

A glucose bolus of 0.3 g/kg of body weight of a 20% glucose solution will be injected intravenously (via an intravenous canula) at time point 0, and subsequent blood samples collected at 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 minutes after the glucose load. Maximum volume of administered 20% glucose solution will be kept at 150 mL. First-phase insulin response (FPIR) will be calculated as the sum of insulin concentrations 2, 4, and 6 minutes after an IV glucose load (13) and as an incremental area under the curve in the first 10 minutes. Insulin sensitivity and glucose effectiveness can be estimated from the 10-60 minute values.

2) HEC

Starting 60 min after the glucose bolus, a HEC will be started with a priming short-acting soluble insulin (Actrapid) rate of 1.5 mU/kg/min for the first 3 min and thereafter a continuous fixed infusion rate of 0.5 mU/kg/min will be used in the remaining 117 min of the study. At the same time, a glucose infusion will be given at the rate to aim to maintain plasma glucose at

5.5 mmol/L. After about 60 min, blood glucose turnover reaches an euglycaemic steady state which is maintained until the end of the clamp (i.e. for another 60 min). Insulin sensitivity is represented by M-value defined as average glucose infusion rate during the euglycaemic steady state (expressed in milligrams of glucose per kilogram of body weight per minute), with higher M-value reflecting better insulin sensitivity. In addition, sensitivity index will be calculated as a ratio between M-value and mean insulin concentration during the euglycaemic steady state.

At the end of studies, participants will be given a meal before leaving the research facility.

Glucose concentration will be measured using a YSI analyser. Insulin concentrations will be determined in the Core Biochemical Assay Laboratory of the Cambridge University Hospitals. Full blood count, urea and electrolytes, liver function tests, and HbA1C will be analysed in the Cambridge University Hospitals laboratory. Plasma samples will be stored for the possible additional analyses. Total bleed volume is expected to be approximately 150 mL.

4.4 Definition of End of Study

The date of last visit of the last participant will constitute the end of the study. No follow-up visits are planned.

5. SAFETY REPORTING (IF APPLICABLE)

5.1 Definition of Serious Adverse Events

The ICH Guideline for Good Clinical Practice E6 (R1) defines an adverse event (AE) as: any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily 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 use of a study medication, whether or not considered related to the study medication.

An abnormal laboratory finding which requires medical intervention by the investigator, or a finding judged by the investigator as medically significant should be reported as an AE. If clinical sequelae are associated with a laboratory abnormality, the diagnosis or medical condition should be reported not the laboratory abnormality. Abnormal laboratory values that are not, in the investigator's opinion, medically significant and do not require intervention should not be reported as AEs.

Definition of Serious Adverse Events (SAE)

An SAE is any AE that:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in offspring of the subject
- Is an important medical event that may jeopardize the participant or may require medical intervention to prevent one of the outcomes listed above

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in this situation. Examples of medically important events are intensive treatment in an emergency department or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalisations.

More than one of the above criteria can be applicable to one event. Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. Medical judgement should be exercised in deciding whether an adverse event or reaction is serious in other situations.

Important adverse events or reactions that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

The definitions of causality categories will be used according to Table 1.

Table 1 Causality categories according to the WHO (Reference: WHO-UMC Causality Categories)

Intensity	Definition
Not assessable	A report suggesting an adverse event, which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

Unlikely	A clinical event, including laboratory test abnormality, with a temporal relationship, which makes a causal relationship improbable, and in which other drugs/treatments, chemicals or underlying disease(s) provide plausible explanations.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the treatment/use of investigational treatment/device, but which also could be explained by concomitant diseases or other drugs/treatments or chemicals.
Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the treatment/use of medical method/device, unlikely to be attributable to concomitant disease(s) or other drugs/treatments or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
Certain	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to study treatment/use of medical method/device and which cannot be explained by concomitant disease(s), other drugs/treatments or chemicals. The response to withdrawal of the treatment (de-challenge) should be clinically plausible. The event must be unambiguous, either pharmacologically or as phenomenon, using satisfactory re-challenge procedures if necessary.

5.2 Reporting Procedures for Serious Adverse Events

All AEs will be recorded in participants medical notes on EPIC (electronic healthcare record used in the Cambridge University Hospitals NHS Foundation Trust). If an AE evolves into a condition that meets the regulatory definition of ‘serious’, it will be reported on the SAE Report Form and submitted to the Sponsor. AEs and SAEs will be collected until the end of the study. If an AE/SAE occur at the last visit, the outcome of these will be followed-up as clinically indicated.

When reporting adverse events, all pertinent data protection legislation must be adhered to.

The serious adverse event report should contain the following information:

1. Study identifier

2. Participant's unique study number

3. Date of birth

4. Event description

5. Start date of event

6. Laboratory tests used and medical interventions used to treat the SAE

7. Planned actions relating to the event, including whether the study device was discontinued

8. Statement on the patient's current state of health

9. Reason for seriousness (i.e. death, life threatening, hospitalisation, disability/incapacity or other)

10. Evaluation of causality (including grade of relatedness) with the following (more than one may apply):
a. the investigational treatment

b. the clinical study/a study specific procedure

c. other: e. g. concomitant treatment, underlying disease
11. Reporter's name, date and signature

If the above information is incomplete at the time of initial reporting, all appropriate information should be provided as soon as this becomes available.

The relationship between the study medication and SAE should be assessed by the investigator, as should the anticipated or unanticipated nature of any SAEs. All SAEs whether or not deemed related to the study medication and whether anticipated or unanticipated must be reported to the Sponsor by email or fax within 24 hours (one working day) of the Investigator learning of its occurrence.

6. STATISITICS

6.1 The Number of Participants

Since this is a feasibility study, formal sample size calculations are not required at this stage. However, we searched previous reports of the studies using Botnia clamps to obtain a range of standard deviations (SD) of the primary outcome, M-value (defined as average glucose infusion rate when euglycaemic steady state has been reached after the start of insulin infusion, and is expressed in milligrams of glucose per kilogram of body weight per minute).

Considering the statistical power of 80%, α=0.05, desired increase in M-value (improvement in insulin sensitivity) of 15% (assumed pre-treatment and post-treatment means are shown below), SD for M-values of 1.5 mg/kg*min (chosen based on the reported or calculated SD in previous studies with SD ranging from 1.2 to 2.5 mg/kg*min (13, 15-18)) and within-person correlation coefficient of 0.7 (calculated based on the reported data of previous study using Botnia clamp in healthy volunteers (18)), the sample size required is N=10. Accounting for a possible dropout/ technical failure rate with clamps / cannulation etc. of 20%, the target sample size is 12 (i.e. 24 Botnia clamps in total). We will allow for the replacement of up to 5 recruits if they are deemed unable to study (e.g. poor venous access). Analysis will be performed in STATA version 16 (14).

6.2 Sampling

A simple random sample of healthy adult volunteers will be used to maximise the representativeness of this population stratum. We are going to use poster advertisements in the Wolfson Diabetes and Endocrine Clinic. In addition, the study will be advertised through social media.

6.3 Analysis of Endpoints

Continuous variables will be shown as mean and standard deviation (SD) for normally distributed variables or median and interquartile range (IQR) when there is a departure from normality. Primary outcome, M-value, will be shown as mean and standard error of the mean (SEM) for comparability with previous literature. Insulin secretion will be expressed in units per kg of total body weight. First phase insulin response will be calculated as an incremental trapezoidal area under the curve in the first 10 minutes of the intravenous glucose tolerance test component of the Botnia clamp. Categorical variables will be shown as N (%). The study is powered for the primary outcome being M-value (mg/kg*min). Paired t-test will be used to test the differences in M-values between sumatriptan and placebo study days. Logarithmic

transformations will be applied as appropriate to approximate normal distribution. All tests will be two-sided with statistical significance defined as $p<0.05$. Data will be collected at the screening visit and subsequent 2 visits by the principal investigator and research nurses. The principal investigator will analyse data at the end of the study. Microsoft Excel and STATA (version 16)(14) will be used for analysis.

7. ETHICAL AND REGULATORY COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice and the Declaration of Helsinki. The participants may not have direct short-term benefits from taking part in the study which is explained in the PIS. However, the results of this study will help design future studies focused on people with diabetes and open new research avenues and ultimately clinical benefits of adjunct treatment to insulin (T1D) and/or another medication in the treatment of T2D. The risks are described in detail in the PIS and can be broadly classified into procedure related risks (PIS) and side effects of sumatriptan (Appendix 4; PIS). Participants will keep a copy of the PIS and ICF while another copy will be kept in the Investigator Site File and the participants medical records. Strategies taken to minimise these risks are also described in the PIS and the Background section of this Protocol. The study will be conducted on overweight otherwise healthy volunteers as this is the first study in humans investigating the effects of sumatriptan on glucose homeostasis independent of weight loss and allows an assessment of insulin secretion and sensitivity. We have chosen the most parsimonious design and method to minimise the burden to participants. Data collection methods involve measurement of vital and anthropometric parameters as well as frequent blood sampling, and participant dignity will always be maintained throughout the study.

7.1 Data Protection and Patient Confidentiality

All data will be processed in accordance with the General Data Protection Regulation (GDPR). Electronic data will be stored on password-protected computers. All paper records will be kept in locked filing cabinets, in a secure office. Only members of the research team will have password access to the anonymised electronic data. Only members of the research team will have access to the filing cabinet. Paper copies of the data will be stored for 5 years in line with the Data Protection Act 2018. The investigators and the rest of the study team will ensure that personal information of the participants is always protected. In the correspondence with the Sponsor confidentiality and anonymous nature of the data will be preserved by using unique participant identifiers (study numbers) instead of the names.

All samples will be labelled using a unique study ID which is not identifiable outside the clinical study team. Residual blood samples will be stored for potential future analysis in ethically approved studies for up to 5 years.

Participants will be able to send questions, comments and requests about their personal data to Michelle Ellerbeck (Data Protection Officer at Cambridge University Hospitals) at gdpr.enquiries@addenbrookes.nhs.uk.

7.2 Indemnity

The clinical investigators are indemnified to cover negligent harm to patients participating in the study by their membership of medical defence organisations.
The Sponsor (Cambridge University Hospitals NHS Foundation Trust) has insurance to cover the costs of illness or injuries arising from the design, management and conduct of the study.
The Sponsor has made no arrangements for payment of compensation in the event of harm to the research participants where no legal liability arises.

8. DISSEMINATION POLICY

The Sponsor (Cambridge University Hospitals NHS Foundation Trust) owns the data arising from this study. Upon completion of the study, the data will be analysed and tabulated and the Final Study Report will be prepared. The Final Study Report will be kept together with other study documents and a copy will be sent to the Funder (DRWF) as specified in the Terms and Conditions of the award and to the Research and Development Office of the Sponsor. The DRWF will be acknowledged in the publications arising from this project but have no review or publication rights *per se*.

Data will be published in internationally peer-reviewed scientific journals. The investigators (Dr Golubic and Dr Evans) and other members of the study team will be co-authors. The privacy of each subject and confidentiality of their information shall be preserved in reports and publication of data. There are no time limits to publish the data but the investigators will endeavour to submit the first scientific report as soon as possible after the analysis is completed.

The participants will be notified of the outcome of the study in a form of a lay summary of the results and their meaning for future research and people with diabetes after the first scientific report has been published. The participants are welcome to request the detailed report directly from the PI.

The study protocol and full study report will be part of the publications and therefore available publicly. Direct access to the individual level source data will be provided for audits, Ethics

Committee review and regulatory authority inspections during and after the study. The fully anonymised data may be shared with third parties for the purposes of advancing management and treatment of diabetes. Appropriate procedures agreed by the investigators will be put in place for data review, database cleaning and issuing and resolving data queries.

9. APPENDICES

Appendix 1 Schedule of Procedures

	When?		
	Screening visit	**Visit 1**	**Visit 2**
What happens?			
Eligibility review	**x**	**x**	**x**
Informed consent	**x**		
Medical history	**x**		
Physical examination	**x**		
Weight measurement	**x**	**x**	**x**
Height measurement	**x**		
Vital signs	**x**	**x**	**x**
Adverse event review		**x**	**x**
Concomitant medication review	**x**	**x**	**x**
Fasted (10 hours)		**x**	**x**
Blood sample collection	**x**		
Pregnancy test (women only; urine sample)	**x**	**x**	**x**
Substance abuse screen (urine sample)	**x**	**x**	**x**
Study drug administration		**x**	**x**
Botnia clamp study		**x**	**x**

Appendix 2 Study timeline

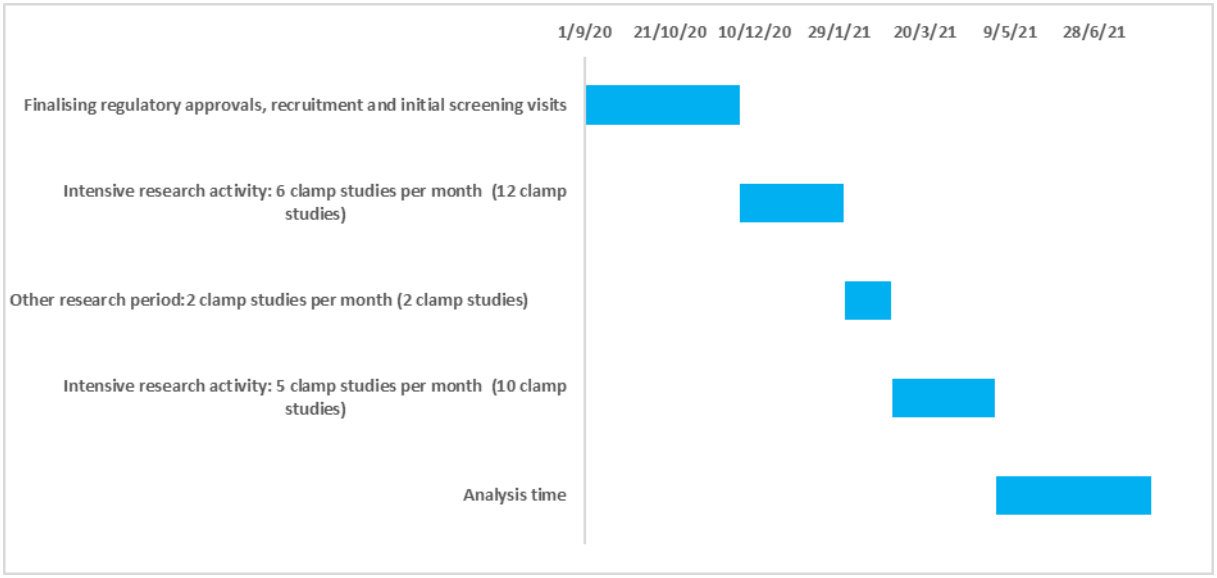

Figure 1 Project timeline

NB Periods of intensive research activity are timed with an academic/research block of the principal investigator which is part of combined clinical and academic training in diabetes and endocrinology. We are aware that the start of the study may be affected by the state of the COVID19 epidemic.

Schedule of events is presented in Appendix 1.

Appendix 3 Details of power calculation

The following is a power calculation for the cross-over trial described in this proposal comparing the effects of a single dose of sumatriptan vs placebo on insulin sensitivity expressed as M-value (mg/kg*min) using paired samples t-test.

α=0.05 and power 80% were set conventionally.

Assumed pre-treatment mean for M-value: 8.0 mg/(kg*min)

Assumed post-treatment mean for M-value: 9.2 mg/(kg*min), i.e. 15% improvement in insulin sensitivity

r=0.7- calculated within-person correlation coefficient

SD=1.5 mg/(kg*min)- assumed standard deviation for pre-treatment and post-treatment M-value

The above parameters yield n=10 for the paired samples t-test.

Accounting for 20% attrition the target n is n=10/0.8=12

Further details:

1) Within-person correlation

Pre-treatment mean for M-value of 8 mg/(kg*min) was chosen based on the reported estimate in Wilms *et al*. using Botnia clamps in healthy volunteers: this was the estimate for the control group (18). The desired improvement in insulin sensitivity is 15% and therefore, the assumed mean for post-treatment M-value is 9.2 mg/(kg*min).

The r (within-person correlation coefficient) was calculated from the reported estimates of SE (and calculated SD) for M-value and n in the study by Wilms *et al.* (18). The following formula was used (19):

$Corr= (SD_E^2 + SD_C^2 – SD_{diff}^2)/(2*SD_E*SD_C)$

Inserting the calculated SD from the reported estimates in the formula above yields r=0.7.

2) Assumed standard deviation for pre- and post-treatment mean M-value

Several previous studies reported SE or SD for M-value. When only SE was available SD was calculated as $SD=SE * n^{1/2}$. The reported or calculated SD in previous studies ranged from 1.2 to 2.5 mg/kg*min (13, 15-18). We arbitrarily chose SD=1.5mg/kg*min for pre-treatment and post-treatment M-value to represent a biologically plausible SD in this study and used it in the calculation of the target n for paired samples t-test.

Appendix 4 Side effects of sumatriptan

Adverse events are listed below by system organ class and frequency. Frequencies are defined as:

- Very common (≥ 1/10)
- Common (≥1/100 to <1/10),
- Uncommon (≥1/1,000 to <1/100),
- Rare (≥1/10,000 to <1/1,000),
- Very rare (<1/10,000), not known (cannot be estimated from the available data).

Some of the symptoms reported as undesirable effects may be associated symptoms of migraine.

Immune system disorders

Not known: hypersensitivity reactions ranging from cutaneous hypersensitivity (such as urticaria) to anaphylaxis.

Nervous system disorders

Common: dizziness, drowsiness, sensory disturbance including paraesthesia and hypoaesthesia

Not known: seizures, although some have occurred in patients with either a history of seizures or concurrent conditions predisposing to seizures. There are also reports in patients where no such predisposing factors are apparent; tremor, dystonia, nystagmus, scotoma

Eye disorders

Not known: flickering, diplopia, reduced vision. Loss of vision including reports of permanent defects. However, visual disorders may also occur during a migraine attack itself.

Cardiac disorders

Not known: bradycardia, tachycardia, palpitations, cardiac arrhythmias, transient ischaemic ECG changes, coronary artery vasospasm, angina, myocardial infarction

Vascular disorders

Common: transient increases in blood pressure arising soon after treatment, flushing

Not known: hypotension, Raynaud's phenomenon

Respiratory, thoracic and mediastinal disorders

Common: dyspnoea

Gastrointestinal disorders

Common: nausea and vomiting occurred in some patients, but it is unclear if this is related to sumatriptan or the underlying condition.

Not known: ischaemic colitis, diarrhoea

Musculoskeletal and connective tissue disorders

Common: sensations of heaviness (usually transient and may be intense and can affect any part of the body including the chest and throat), myalgia

Not known: neck stiffness, arthralgia

General disorders and administration site conditions

Common: pain, sensations of heat or cold, pressure or tightness (these events are usually transient and may be intense and can affect any part of the body including the chest and throat); feelings of weakness, fatigue (both events are mostly mild to moderate in intensity and transient)

Investigations

Very rare: minor disturbances in liver function tests

Psychiatric disorders

Not known: anxiety

Skin and subcutaneous tissue disorders

Not known: Hyperhidrosis

Source: Electronic Medicines Compendium (10)

Appendix 5 Glossary

AE- adverse event

BMI- Body Mass Index

CUH- Cambridge University Hospitals

DRWF- Diabetes Research and Wellness Foundation

FDA- Food and Drug Administration

FSivGTT- frequently sampled intravenous glucose tolerance test

HEC- hyperinsulinaemic euglyceamic clamp

ICF- Informed Consent Form

IQR- interquartile range

PI- Principal Investigator

PIS- Participant Information Sheet

REC- Research Ethics Committee

SAE- serious adverse event

SD- standard deviation

SE- standard error

SEM- standard error of the mean

TRF- Translational Research Facility

T1D- type 1 diabetes

T2D- type 2 diabetes

WHO- World Health Organization

5HT- serotonin

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