University of Exeter

Royal Devon University Healthcare NHS Foundation Trust

FULL TITLE OF THE STUDY:

Stimulated glucagon as a biomarker of hypoglycaemia risk in Type 1 diabetes

SHORT STUDY TITLE / ACRONYM: MUGGLE

RESEARCH REFERENCE NUMBERS AND DATES IRAS REC SPONSOR Protocol Reference	 **319581** **22/LO/0719** **London – Hampstead REC** **2021-22-59**
TRIAL REGISTRY NUMBER AND DATE ClinicalTrials.gov	 **NCT 05616273**
PROTOCOL VERSION NUMBER AND DATE	**2.0** 04 November 2022
FUNDER & REF	The Leona M. and Harry B. Helmsley Charitable Trust Type 1 Diabetes Program Grant #2012-04188
OTHER RESEARCH REFERENCE NUMBERS Clinical Research Facility (CRF)	 **CRF 481**

This protocol has regard for the HRA guidance

KEY STUDY CONTACTS

CHIEF INVESTIGATOR	**Professor Richard Oram** Diabetes UK Harry Keen Fellow Associate Professor & Honorary Renal Consultant, University of Exeter Medical School, Faculty of Clinical & Biomedical Sciences, RILD Level 3, Barrack Road, Exeter EX2 5DW, UK Mobile: +44 (0) 7792 484421 Email: R.Oram@exeter.ac.uk
CO-INVESTIGATORS	**Professor Andrew Hattersley** Professor of Molecular Medicine University of Exeter Medical School, Faculty of Clinical & Biomedical Sciences, RILD Level 3, Barrack Road, Exeter EX2 5DW, UK Tel: +44 (0) 1392 408260 Direct Mobile: +44 (0) 7973 165924 PA Tel: +44 (0) 1392 408231 Email: A.T.Hattersley@exeter.ac.uk **Professor Timothy McDonald** Laboratory Director Blood Sciences Royal Devon University Healthcare NHS Foundation Trust & Clinical Professor (NIHR Fellow), University of Exeter Medical School, RILD Level 3, Barrack Road, Exeter EX2 5DW, UK Tel: +44 (0) 1392 408250 / 403699 Email: timothy.mcdonald@nhs.net **Professor Patrik Rorsman** Professor of Diabetic Medicine Oxford Centre for Diabetes, Endocrinology and Metabolism University of Oxford Churchill Hospital Oxford OX3 7LE Email: patrik.rorsman@drl.ox.ac.uk

NIHR Support Infrastructure:	
NIHR Exeter Clinical Research Facility	Tel: +44 (0) 1392 408181 Email: crf@ex.ac.uk
Professor of Cardiovascular Sciences and Scientific Director of NIHR Exeter Clinical Research Facility	**Professor Angela Shore** Professor of Cardiovascular Sciences and Scientific Director of NIHR Exeter Clinical Research Facility NIHR Exeter Clinical Research Facility, RILD Level 2, Barrack Rd, Exeter EX2 5DW Tel: +44 (0) 1392 408181 Email: A.C.Shore@exeter.ac.uk
Statistician	**Dr Beverley Shields** Senior Lecturer in Medical Statistics University of Exeter Medical School RILD Level 3, Barrack Rd, Exeter EX2 5DW Tel: +44 (0) 1392 408203 Email: B.Shields@exeter.ac.uk
Project Manager	**Michelle Hudson** Research Project Manager NIHR Exeter Clinical Research Facility RILD Level 2, Barrack Rd, Exeter EX2 5DW Tel: +44 (0) 1392 408181 Email: M.Hudson@exeter.ac.uk
SPONSOR	**University of Exeter**
SPONSOR REPRESENTATIVE	**Pam Baxter** Research Governance Manager (Health & Social Care) Research Ethics and Governance Office, University of Exeter, Lafrowda House, St Germans Road, Exeter, Devon, EX4 6TL Tel: +44 (0) 1392 723588 Email: P.R.Baxter2@exeter.ac.uk
NHS R&D CONTACT	**Lisa Treeby** Senior Network Research Facilitator R & D Room F5, Bowmoor House, Royal Devon University Healthcare NHS Foundation Trust, Barrack Road, Exeter EX2 5DW Tel: +44 (0) 1392 403814 Email: Lisa.Treeby@nhs.net

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the UK Policy Framework for Health & Social Care, the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

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 clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly 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:

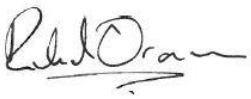
Signature: 

Date: 04/11/2022

Name: Ms Gail Seymour

Position: Research Governance Manager (Health & Social Care)

Chief Investigator:

Signature: 

Date: 27/10/2022

Name: **Professor Richard Oram**

Position: Associate Professor in Diabetes and Nephrology

STUDY SUMMARY/SYNOPSIS

Study Title	Stimulated glucagon as a biomarker of hypoglycaemia risk in type 1 diabetes	
Short Title and Internal ref. no.	MUGGLE (CRF 481)	
Study Design	Prospective observational cohort	
Lead Trial Centre	Royal Devon University Healthcare NHS Foundation Trust	
Study Summary	This programme of work will establish: (i) the relationship between oral and intravenous (IV) mixed meal (MM)-stimulated plasma glucagon and hypoglycaemia, (ii) the underlying physiology of amino acid (AA)-stimulated glucagon secretion and (iii) whether a simple blood glucagon assessment could be a clinically useful biomarker of hypoglycaemia risk and therefore directly inform clinical care of people with type 1 diabetes (T1D), particularly those with highest risk of hypoglycaemia.	
Study Participants	Individuals with long duration type 1 diabetes (identified from TIGI study/research cohorts).	
Planned Sample Size	75	
Treatment duration	N/A Participants will undergo usual clinical care.	
Follow up duration	7 months	
Planned Study Period	23 months: 1st November 2022 – 30th September 2024* *Estimated duration for the study protocol (e.g. from when all approvals have been received [REC, HRA and R&D], 15 months' recruitment, through to when the last subject/participant recruited has completed all study processes after 7 months follow-up, plus 3 months for data completion).	
Main Inclusion Criteria	• Clinical diagnosis of Type 1 diabetes • Insulin treated • Known urine C-peptide (UCPCR) status (positive/negative defined by UCPCR 0.2nmol/mmol cut-off) • Age 16-65 years inclusive • Able and willing to provide informed consent/assent	
	Objectives	**Outcome Measures**
Primary	To validate the relationship of both post-mixed meal and amino acid stimulated glucagon levels to hypoglycaemia frequency in people with long duration T1D.	1. MM & AA-induced plasma glucagon levels. 2. continuous glucose monitoring (CGM) time spent in hypoglycaemia 3. Self-reported hypoglycaemia episodes, hypoglycaemia fear/awareness, and fear of hypoglycaemia.
Secondary	1. Test the longitudinal variability of glucagon and its relationship to episodes of hypoglycaemia in long duration T1D. 2. Establish the optimal sampling, analysis, and storage conditions	1. Stimulated and plasma glucagon levels. 2. continuous glucose monitoring (CGM)

	for glucagon to be conveniently measured in clinic or at home 3. Investigate the cause/effect relationship between hypoglycaemia and alpha cell function	

FUNDING AND SUPPORT IN KIND:

FUNDER(S)	**FINANCIAL AND NON FINANCIAL SUPPORT GIVEN**
The Leona M. and Harry B. Helmsley Charitable Trust Type 1 Diabetes Program Grant # 2012-04188	£693,400
Research & Development Directorate, Royal Devon University Healthcare NHS Foundation Trust	Site-level Support
NIHR Exeter Clinical Research Facility	Support Infrastructure

ROLES AND RESPONSIBILITIES:

Sponsor

The sponsor has no role in the study design or data analysis, interpretation or manuscript writing.
The sponsor will review and approve the study protocol and supporting documents.
The sponsor takes overall responsibility for the study site monitoring that is carried out by the CI's team.

Details of the roles, responsibilities and individuals involved in the Study Management Group and Study Steering Committee can be found in the Trial Master File.

STUDY MANAGEMENT:

Day to day management of the study will be undertaken by the CI and Project Manager, with the support of the NIHR Exeter Clinical Research Facility.

Study Management Group (SMG)

The SMG, to include the CI, Co-investigators, and Project Manager, will meet/communicate regularly to ensure all practical details of the study are progressing and working well.

Study Steering Committee (SSC)

An independent steering committee will be appointed, made up of researchers, physicians and patients, to provide independent guidance and ensure that study progress and the conduct of the study is appropriate.
The steering committee will meet 6 monthly with the CI and members of the study team and Sponsor Representative.

Table of Contents

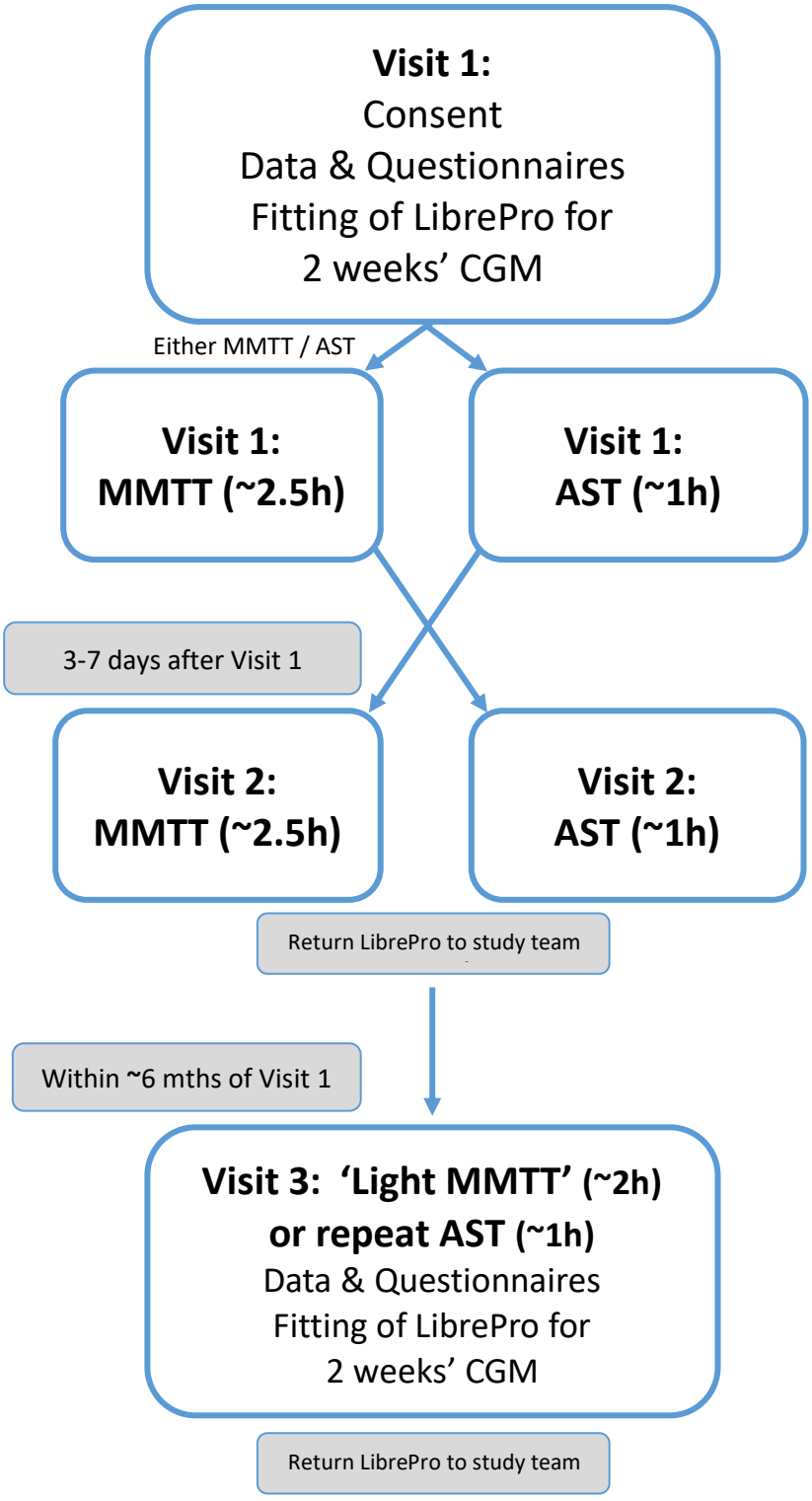
KEY WORDS:

type 1 diabetes	autoimmune diabetes	beta cell (β-cell) destruction
hypoglycaemia	hyperglycaemia	glucagon
type 1 diabetes genetic risk	Alpha cell	

Glossary of Terms and Abbreviations

AE	Adverse Event
AR	Adverse Reaction
AST	Arginine Stimulation Test
CI	Chief Investigator
CRF	Case Report Form / Clinical Research Facility
CRN	Clinical Research Network
DM	Diabetes mellitus
DMP	Data Management Plan
ECRF	Exeter Clinical Research Facility
GCP	Good Clinical Practice
HRA	Health Research Authority
ICF	Informed Consent Form
ISF	Investigator Site File
MHRA	Medicines and Healthcare products Regulatory Agency
MMTT	Mixed Meal Tolerance Test
Non-CTIMP	Non-Clinical Trial of Investigational Medicinal Product
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
Participant	An individual who takes part in a clinical trial
R&D	Research and Development Office
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SIV	Site Initiation Visit
SDV	Source Document Verification
SOP	Standard Operating Procedure
SMG	Study Management Group
SSC	Study Steering Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes

MUGGLE STUDY - SUMMARY FLOW CHART:



MMTT: Mixed Meal Tolerance Test
AST: Arginine Stimulation Test
Light MMTT: shorter MMTT with fewer sampling time points
CGM: Continuous Glucose Monitoring

1. Introduction

1.1 Background

Importance and potential benefit

Type 1 diabetes (T1D) is a common autoimmune disease that causes destruction of beta cells in the pancreas that secrete insulin, a glucose-controlling hormone, leading to high blood glucose (hyperglycaemia).

Hypoglycaemia is a common, dangerous complication of insulin treatment in T1D
Whilst high blood sugar level is a problem for people with Type 1 diabetes (T1D), taking insulin medication to lower sugar levels, delayed meals and exercise can all result in dangerously low blood sugar levels (hypoglycaemia). Hypoglycaemia can lead to unconsciousness or death and is a major obstacle to good management of the disease. The biological causes of hypoglycaemia, and thus ways to prevent it, are poorly understood. In people without diabetes, a hormone called glucagon is secreted by alpha cells in response to low blood glucose, acting to raise blood glucose levels. However, in people with type 1 diabetes, glucagon secretion is commonly impaired during hypoglycaemia.

People with T1D experience an average of two hypoglycaemic episodes per week, and ~30% of people with T1D have at least one episode of severe hypoglycaemia (requiring third party intervention) per year[2]. This potentially life-threatening complication poses one of the largest barriers to achieving tight glycaemic control, in addition to contributing greatly to the daily burden of people with T1D. As such, it is important to recognise individuals at high risk of hypoglycaemia. Strict glycemic control is a risk factor for hypoglycaemia but HbA1c alone only explained 6-12% of variation in severe hypoglycaemia rate in DCCT[3,4]. Other risk factors include disease duration, age and hypoglycaemia awareness, history of hypoglycaemia and absent beta cell function[2]. Identification of individuals at high hypoglycaemic risk is critical for best management and for providing personalised strategies and therapies to reduce hypoglycaemia.

Chief Investigator, Professor Richard Oram, and colleagues have preliminary data suggesting that dysregulation of glucagon is variable in individuals with T1D and that high blood glucagon concentrations after a mixed meal (i.e. a mixed-meal stimulus of protein and carbohydrates) is correlated with reduced hypoglycaemia. This may be because amino acids are an additional alpha cell stimulus that cause glucagon release in a similar manner to hypoglycaemia. Professor Oram, with Professor Patrik Rorsman (University of Oxford) and colleagues, propose a combination of laboratory and clinical studies to understand the relationship between these dysfunctions and determine whether, ultimately, a mixed-meal stimulation of glucagon could predict the risk of hypoglycaemia. In combination with other known risk factors, this biomarker could be used, for example, to stratify patients in clinical trials or to help individuals manage blood glucose levels. This protocol relates to the clinical study element of this grant's programme of work.

Abnormal alpha cell function is a feature of T1D
Glucagon, secreted by alpha cells in pancreatic islets, is the body's principal glucose raising hormone[5]. Hyperglycaemia leads to the suppression of glucagon secretion by a combination of metabolic effects within the alpha cell itself, possibly augmented by activation of paracrine processes (mediated by, for example, insulin/zinc/GABA released by the neighbouring beta and delta cells, respectively)[6]. Hypoglycaemia leads to stimulation of glucagon secretion by the reversal of these effects. The net effect is increased glucagon, which signals the liver to increase glucose output through glycogenolysis and gluconeogenesis. This glucagon counter-regulatory response plays a critical role in protection from hypoglycaemia. In T1D there is significant glucagon dysregulation[7], including fasting and postprandial hyperglucagonemia soon after diagnosis, and an impaired glucagon response to hypoglycaemia[8]. A recent study of organ donors with T1D highlights functional and gene expression abnormalities of alpha cells in T1D but the effects of hypoglycaemia were not explored[9]. In addition, glucagon response appears to become increasingly dysregulated with increasing disease duration and declining beta cell function[10,11]. Whether the progressive

dysregulation of glucagon secretion is a consequence of the loss of paracrine control mediated beta cell products (including insulin), or systemic metabolic defects (such as hyperglycaemia), is unknown.

Plasma glucagon is differentially regulated by ingestion of glucose and a mixed meal (MM) (containing proteins). Whereas increased glucose results in a reduction of plasma glucagon, the response to a mixed meal consists of a transient stimulation that precedes the inhibitory effect[12,13]. It is believed that the initial increase reflects the stimulation of glucagon secretion by the amino acids (AA). Indeed, a cocktail of amino acids is a powerful stimulus of glucagon secretion[14]. Interestingly, the capacity of amino acids (like arginine) to stimulate glucagon secretion is preserved (or even enhanced) in T1D patients[1,15]. An outstanding question is whether post mixed meal increases in glucagon could relate to glucagon directly released from the gut or glucagon released by alpha cells directly stimulated by amino acids, and whether this impacts the activity of glucagon released[16,17]. Even though not in a setting of hypoglycaemia, AA-induced increase in plasma glucagon may therefore potentially provide a means to estimate the maximum functional alpha cell 'reserve'.

There is an abundance of alpha cells and glucagon staining in the pancreatic islet, even in people with long duration T1D, so any potential markers and mechanisms of hypoglycaemia that relate to alpha cell function may be amenable to intervention even if beta cell function is lost. Whilst abnormalities of alpha cell function and glucagon release have been described in groups of people with T1D[1], there are no studies looking at whether responsiveness of alpha cells explains differences in rates of hypoglycaemia between individuals with T1D in these cohorts. Professors Oram and Rorsman expect these studies to reveal the relationship between the under-secretion of glucagon during hypoglycaemia and the counterproductive over-secretion of glucagon after a meal. It is hoped this research will determine the feasibility of using this relationship as a blood test as a clinically useful biomarker of hypoglycaemia risk and therefore directly inform clinical care of people with T1D, particularly those with highest risk of hypoglycaemia.

1.2 Lay Summary

Type 1 diabetes (T1D) results from destruction of insulin producing beta cells by the body's own immune system (autoimmunity) causing an individual to lose the ability to make enough insulin to control their blood sugar levels and need to have insulin injections to lower blood glucose levels. Whilst high blood sugar level is a problem for people with Type 1 diabetes, taking insulin medication to lower sugar levels, delayed meals and exercise can all result in dangerously low blood sugar levels (hypoglycaemia). The biological causes of hypoglycaemia, and ways to prevent it are poorly understood. In non-diabetic individuals, a hormone called glucagon is secreted naturally to raise blood glucose levels but it is unclear why glucagon secretion is impaired during hypoglycaemia in individuals with T1D.

The aim of this work is to study the relationship between a glucagon stimulation test and risk of hypoglycaemia in T1D. It is hoped this research will establish whether this relationship could be used as a blood test and be a clinically useful biomarker of hypoglycaemia risk and, therefore, directly inform clinical care of people with T1D, particularly those with highest risk of hypoglycaemia.

Muggle study website: https://www.diabetesgenes.org/current-research/muggle/

2. Study Objectives and Design

2.1 Rationale for Study Design

Normally blood glucose levels are controlled by insulin, a hormone that lowers high blood glucose levels, and glucagon which is released into the blood to raise glucose when levels are low. For people with Type 1 diabetes (T1D), taking insulin medication to lower glucose levels, delayed meals and exercise can all result in dangerously low blood sugar levels (hypoglycaemia). The ability to release glucagon and correct blood glucose can vary, particularly in people who have had T1D for years. For some people, glucagon levels may not be able to rise quickly, reflecting an impairment in glucagon production. The study aims to find out how blood glucagon levels after a stimulus compare to a person's own experience of hypoglycaemia, and hypoglycaemia as measured by flash glucose monitoring in T1D. We hope this research will establish whether glucagon measured after a meal

could be used as a blood marker of hypoglycaemia risk and help to identify individuals at high risk, potentially identifying some people who could benefit from treatments targeting glucagon.

2.2 Hypothesis:

Low post-mixed meal (MM) stimulated glucagon secretion is associated with impaired alpha cell function and predicts hypoglycaemia.

2.3 Study Aims

i) To understand the relationship of stimulated glucagon secretion to hypoglycaemia, a hormone that raises blood glucose levels, in Type 1 diabetes (T1D).
ii) To create a blood test to predict the risk of hypoglycaemia (low blood glucose levels).

Primary Objective

To clinically validate the relationship of both post mixed-meal and amino-acid stimulated (arginine stimulation test, AST) glucagon levels to hypoglycaemia frequency in people with long duration T1D by using continuous glucose monitoring (CGM) and hypoglycaemia questionnaire data.

Secondary Objectives

1. Test the longitudinal variability of glucagon levels and its relationship to episodes of hypoglycaemia in long-duration T1D (repeated stimulation and inhibition experiments within isolated islets and individuals (laboratory and clinical studies);
2. Derive a mechanistic understanding of the relationship between plasma glucagon levels under basal conditions, following a stimulation (by mixed meal or arginine)

Primary Endpoint/Outcome

The outcome of this programme of work will be a description of the strength and reproducibility of the relationship of post-MM and AST glucagon with hypoglycaemia. We will describe longitudinal stability of stimulated glucagon, its relationship with variation in hypoglycaemia frequency, and the potential to predict future hypoglycaemia.

Secondary Endpoints/Outcomes

We will describe the combined predictive power of glucagon and clinical variables to predict future hypoglycaemia risk, facilitating creation of a simple clinical prediction tool to predict hypoglycaemia.

/Continued

2.4 Study Timeframe:

Milestones		*Yr1* *Nov 22 – Oct 23*	*Yr2* *Nov 23 – Sept 24*	
	5 m *June-Oct 22*	1-12 m *Nov 22-Oct 23*	13 –17m *Nov 23-Mar 24*	18 – 23m *Apr-Sept 24*
Ethical/Governance approvals & work-up				
Recruitment				
Follow-up tests				
Data collection				
Sample batched analysis				
Data analysis				
Writing up/future funding applications				
Writing up/future funding applications				

2.5 Study Design

This is a prospective observational cross-sectional study. Please refer to the study Summary Flow Chart (page 11).

This programme of work will establish:

(i) the relationship between oral and intravenous (IV) stimulated plasma glucagon and hypoglycaemia.
(ii) the underlying physiology of amino acid (AA)-stimulated glucagon secretion.
(iii) whether a simple blood glucagon assessment could be a clinically useful biomarker of hypoglycaemia risk and therefore directly inform clinical care of people with type 1 diabetes (T1D), particularly those with highest risk of hypoglycaemia.

2.6 Study Setting

The Muggle study is a prospective observational study, operating in secondary care. This is a single-site study and participants will be recruited by the NIHR Exeter Clinical Research Facility.
The Exeter Blood Sciences Laboratory is an NHS centre of excellence for diagnostic tests.

3. Subject Selection

3.1 Number of Subjects and Subject Selection

Study cohort:
75 individuals with long duration T1D, identified from participation in the TIGI study (UK IRAS ID**:** 141756, REC Number: 13/SW/0312), with consent to be contacted for future research.

The TIGI participants have a clinical diagnosis of T1D, were on insulin from diagnosis and, if BMI was >30, were autoantibody positive. We will aim to recruit 50 people with undetectable C-peptide and 25 with detectable C-peptide. The selection of people with absent C-peptide allows a secondary analysis focussed on stimulated glucagon without any potential interaction with endogenous insulin secretion. It also focusses on a group of people known to be at high hypoglycaemia risk due to lack of endogenous insulin.

3.2 Inclusion Criteria

- Clinical diagnosis of Type 1 diabetes
- Insulin treated
- Known urine C-peptide status (using Urinary C-Peptide Creatinine Ratio [UCPCR], positive/negative defined by UCPCR 0.2nmol/mmol cut-off)
- Age 16-65 years inclusive
- Able and willing to provide informed consent/assent.

3.3 Exclusion Criteria

- Age less than 16 year or over 65 years
- Pregnant or lactating (as this may limit blood sampling and affect T cell function)
- Any medical condition that, in the opinion of the investigator, would affect the safety of the subject's participation, or validity of results.

4. Study Procedures

Please refer to the flow chart on page 11 for a summary of the study procedures.

The study procedures described below will be conducted in accordance with detailed SOPs/Work Instructions provided by the CI.

4.1 Research Visits

Recruitment will be facilitated within the Exeter CRF.

The potential participant's previous laboratory results, gained during participation of the TIGI study, will be reviewed to confirm eligibility to the study. The potential participant will be written to and provided with the study information leaflet and invited to respond indicating their interest in participating, prior to the research nurse contacting them to discuss the study in detail and arrange an appointment.

Participants will be invited to attend the NIHR Exeter CRF for their appointments (1 to 3.5 hours depending on visit) having fasted overnight (from 10 pm the night before).

All 75 participants will undergo two visits within the first week of recruitment, a Mixed Meal Tolerance Test (MMTT) visit and an Arginine Stimulated Test (AST). A 'light MMTT' or second AST will be performed at a visit approximately 6 months later.

4.2 Informed Consent Procedures

All participants recruited to the study will be required to give written informed consent prior to any data collection or procedure. Prior to consent, all potential participants will be provided with detailed written information about the study (that complies with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018) and an opportunity to discuss it with one of the research team. As stipulated by GCP, participants will be provided with adequate time to consider giving consent. It is the responsibility of the PI, or an appropriately trained and delegated individual, to obtain written informed consent from each participant following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. Informed consent should not be taken until the participant is content that all questions have been adequately answered.

All participants will be informed of their right to withdraw from the study at any time without prejudice or jeopardy to any future clinical care.

A unique study ID will be allocated to the participant to link all participant study information and samples. The participant's clinical characteristics and sample results will be recorded on the participant's Case Report Form and entered on the study database. The recruiting site will retain the original Consent Form and CRF, to be filed in the Investigator Site File.

4.3 Core data collection, measurements and tests

All participants will undergo core data collection, measurements and provide fasted and stimulated blood samples via a cannula at the baseline MMTT and AST visits 1 and 2 and again at the 6 months

'Light MMTT' / repeat AST visit. The order of MMTT and AST tests in visits 1 and 2 will be randomised to check for an order effect. Test choice for Visit 3 depends on the peak glucagon results from Visits 1 and 2.

Core data collection and measures:
Height (m), weight (kg), age of diagnosis, current and previous insulin treatment and family history of diabetes will be recorded for all participants.

At Visits 1 and 3, participants will be asked to complete the hypoglycaemia questionnaire data (Clarke and Gold scores) to assess awareness of hypoglycaemia and quality of life measures which may relate to the MM-glucagon. Participants will be given an Abbot FreeStyle Libre Pro[27] continuous blood glucose monitor (CGM) and asked to perform a 2-week continuous measure of blood glucose (CGM). We will not change clinical diabetes care and participants with T1D will use their own insulin regimes and self-monitoring (even if CGM or Abbot FreeStyle Libre Pro) during the study.

Tests and blood samples:
Visits 1 and 2 will be approximately 3-7 days apart. The order of test will be randomised to check for a test order effect. Visit 3 will be approximately 6 months after Visit 1 and the test conducted will be determined by the peak glucagon results from Visits 1 and 2.

A small cannula (thin plastic tube) will be inserted into a vein, following standard clinical practice, for the duration of each visit to facilitate repeat sampling with minimal invasion, discomfort and potential trauma from repeat blood sampling. For the arginine stimulation test (AST), a cannula will also be inserted into a vein in the non-sampling arm to administer the arginine bolus.

Fasted samples will be obtained for baseline measures: routine glucose and HbA1c, plasma and serum for storage and future batched analysis including measures of glucose, glucagon, insulin (C-peptide) and other markers of interest, plus sodium heparin and PAXgenesRNA samples for T1D immune function analysis.
Stimulated samples (see test descriptions below): insulin (C-peptide), glucose and glucagon will be measured at all time points.
Spare aliquots from all time points will be saved and stored at -80^{o}C for batched analysis of other biomarkers if required.

The exact number of blood samples may vary but the total amount of blood we will collect at each visit will not exceed 150 millilitres (ml).
At the end of each test period, the participant's blood glucose will be checked and appropriate advice will be given relating to insulin dosage adjustments. The cannula(s) will be removed prior to a light meal being provided.

A) Mixed Meal Tolerance Test (MMTT) at Visit 1 or 2 (approx 2½ hours)
Rationale: The MMTT will provide confirmatory analysis of post-mixed meal glucagon stored in BD P800 and EDTA plasma samples at a range of standard MMTT time points (0, 30, 60, 90 and 120 min) in 75 people. Glucose will be measured in Fluoride Oxalate samples at the same time points.

Participants will:

- be monitored closely throughout their visit for any discomfort or problems and appropriate advice/support will be given if required.
- be given Ensure HP or Fortisip (6ml/kg to a max of 360mls)
- have blood samples collected, to measure insulin, C-peptide, glucose, and glucagon, at specific intervals: -10, 0*, 30, 60, 90, 120* minutes post meal

At the end of the MMTT procedure, the participant's blood glucose will be checked and appropriate advice will be given relating to insulin dosage adjustments prior to a light meal being provided.

B) Arginine Stimulation Test (AST) Visit 1 or 2 (approx 1 hour)
Rationale. An MMTT (despite being convenient because it avoids the need for an IV stimulus) has limitations, including differences in gastric emptying, uptake and effects on enteroendocrine secretion. Therefore, we will compare responses during the MMTT with those obtained during an intravenous arginine challenge, Arginine Stimulation Test (AST), (5 g Arginine during 1 min) at a separate visit from the MMTT visit. Glucose, plasma glucagon and C-peptide will be measured at baseline and specific intervals (see below) minutes following the Arginine bolus injection. This test is designed primarily to test the maximum insulin secretion capacity of a participant's beta cells.

The AST test involves:
1. Set up. Intravenous catheters will be inserted into antecubital veins in both arms. One arm will be used for infusion of Arginine (amino acid), and the other arm for intermittent sampling. Blood glucose concentration will be monitored with regular YSI measures throughout the test.
2. AIRarg (acute insulin response to arginine). Baseline samples will be taken at -10 and 0 minutes. A maximally stimulating dose of Arginine Hydrochloride (5 g) will be injected intravenously for 45s. Samples will be collected at 2, 5, 10, 15 and 30 minute intervals following the Arginine bolus.
3. At the end of the AST procedure, the participant's blood glucose will be checked and appropriate advice will be given relating to insulin dosage adjustments prior to a light meal being provided.

C) 'Light MMTT' – Visit 3, approx 6 months after Visit 1 (2 hours)
The participants will be invited to the research facility approximately 6 months after their first visit to undergo a 'Light MMTT'. This test is as described above for MMTT but we will only collect a single sample at the most informative time, as assessed from the first MMTT and AST. We will repeat clinical data collection, hypoglycaemia questionnaires, 2-week CGM data collection with participant blinded *Abbott FreeStyle Libre Pro*.

At Visit 3, the choice of test will be either a 'Light MMTT' or a repeat AST and will be determined by the peak glucagon results from the tests conducted at Visits 1 and 2.

4.4 Data Collection and Recording

All participants will be assigned a unique study ID. All data and samples collected will be recorded and stored under this ID number. Data will be initially recorded using a study-specific case report form (CRF), with hard copies stored in the Trial Master File (TMF). Anonymised research data will be recorded on a study-specific database. Exeter CRF data quality procedures require data collected and recorded to be screened and reviewed for discrepancies and missing data prior to analysis.

Baseline data to be collected will include:

- Personal identifiers
- Physical measures, including BMI
- Diabetes & other significant medical history
- Medication history
- Blood biochemistry

Permission will be obtained to access medical notes should this be required for diabetes data relevant to taking part in the project.

4.5 End of Study Definition

The parameter marking the end of the study is the 3 months after the final participant's 7 months follow-up period to allow for final collection of data.

4.6 Subject Withdrawal

Subjects will be informed they are free to withdraw from the study at any time up to and including data and sample analysis, without prejudice or jeopardy to any future clinical care. If a patient permanently withdraws from the study, or is lost to follow up, the reason will be recorded.

Criteria for premature withdrawal from the study:

- Participant withdrawal of consent

- Investigators' discretion that it is in the best interest of the participant to withdraw
- Termination of the study by the trial sponsor.

4.7 Data Collection and Follow-up for Withdrawn Subjects

Where a participant withdraws, data will be collected up to the point of withdrawal in line with the protocol and study SOPs. If appropriate, the PI or delegated member of the research team will follow-up with the participant to ensure wellbeing and that ongoing clinical care is unaffected.

Where a participant has prematurely withdrawn but not revoked consent, data and samples will remain within the study and included in any analysis. Where consent is withdrawn, study samples and data will be kept/destroyed as per the following options in line with data SOPs and local guidelines:

- samples and data remain
- samples withdrawn, data remains
- samples remain, data withdrawn
- both samples and data withdrawn.

Withdrawal will be recorded on the study database and the participant's choice of the above options recorded. A withdrawn participant is not obliged to give a reason for withdrawing their consent but, where they are willing to do so, the reason will be documented. Details of all withdrawn participants will be regularly reviewed to enable any study-wide trends relating to study procedures to be ascertained and acted upon where appropriate.

5. Samples

5.1 Sample Collection / Labelling / Logging

All samples will be collected by a suitably qualified and trained member of the research team, whose role is documented on the study delegation log. A detailed SOP/work instruction will be provided detailing the clinical procedure for collecting samples and the logistics of sample labelling, logging and management. All study-specific procedures will be covered during the Site Initiation Visit.

5.2 Laboratory Assessments and Results Reporting

The Exeter Clinical Laboratories have an established pipeline for receiving and processing all research samples, including documentation of chain of custody. Central laboratory analysis of the study samples will be undertaken at the Exeter Blood Sciences Laboratory at the Royal Devon University Healthcare NHS Foundation Trust site. Upon receipt, the specimens will be assessed for content and integrity. The samples will be logged immediately, and all samples will be tracked using the laboratory information management system (LIMS).

All analyses are routine biochemistry tests available in the NHS test repertoire. All assays are CE marked, fully validated and accredited by CPA (Clinical Pathology Accreditation).

The Exeter Blood Sciences Laboratory will provide the Site PI with the results of HbA1c, C-peptide, and glucose within 21 days of receipt of the sample. Results of tests analysed in Exeter will also be uploaded directly to the study database within 21 working days of consent date. Surplus plasma and serum will be stored pending consent and will be destroyed if consent is not given within 3 months of sample receipt. Stored plasma, serum, fluoride oxalate, PAXgeneRNA, and P800 will be used for batched analysis of biomarkers such as glucagon, proinsulin, glucose, RNA, and other factors associated with T1D (e.g. immune system).

5.3 Stored samples

Surplus samples for storage should be processed, logged and frozen at -80°C within 24 hours of receipt. All saved samples will be stored under the study ID, with the file linking the study code to personal identifiable information held securely by the Principal Investigator, accessible only to personnel with training in data protection who require this information to perform their duties. Those with access to personal identifiable data will be documented on the study Delegation Log.

All samples sent to the Exeter Clinical Laboratories must be logged on the study sample database. The Study ID and barcode set will provide a robust pseudo-anonymised system for management of all samples. The research team will monitor consent status via the study database. Samples without consent to store will be destroyed within 3 months of collection. Where samples are unable to be collected, this should be documented under the participant Study ID with reason for non-collection provided. At the end of the study, samples without consent to gift to the Peninsula Research Bank (PRB) will be destroyed.

6. Safety, Definitions and Reporting

6.1 Risks

The blood samples will be collected by members of the research team trained in venepuncture. Any potential discomfort or side-effects will be equivalent to that experienced in routine clinical care. During the MMTT/AST test, there is a risk of hyperglycaemia (from having a meal without the participants' usual mealtime insulin) but risks will be minimized by participants being closely monitored during and after their visit by the diabetes specialist research team. Appropriate advice/support will be given if required.

6.2 Benefits

In the short term, we hope to gain an improved understanding of participants' hypoglycaemia risk. In the longer term, this work may provide evidence that the ability of alpha cells to release glucagon after a mixed meal is variable within people with T1D, associated with hypoglycaemia risk, and could be a biomarker for hypoglycaemia risk.

6.3 Definitions and reporting of adverse effects (what should be reported?)

The timeframe for recording SAEs will be from the time of consent to one week following the last visit of a study subject. Any reportable adverse effects noted will be reported within 24 hours to the CI and the Sponsor as per standard NHS R&D protocols. Nominated co-investigators will be authorised to sign the SAE forms in the absence of the CI at the co-ordinating site, or the PI at the participating sites. *The Sponsor should be informed of these nominated individuals***.**

6.4 Serious Adverse Event (SAE)

An SAE fulfils at least one of the following criteria:

- Is fatal – results in death (NOTE: death is an outcome, not an event)
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect.

Reportable SAEs
ANY hospitalisation relating to diabetes management, including DKA and serious hypoglycaemia.

All reportable SAEs will be recorded in the subject's medical notes, the local Investigator Site File, and the Sponsor SAE form and reported to the Sponsor and CI/central coordinating team, within 24 hours of the CI or PI or co-investigators becoming aware of the event.

All non-reportable SAEs will be recorded in the subject's notes, and the CRF, but the Sponsor SAE form will <u>not</u> be filled out or reported to the Sponsor and CI/central coordinating team.
The following SAEs will be considered recordable but **not reportable** to the Sponsor:

- Hospitalisation for elective treatment
- Hospitalisation for treatment of a pre-existing condition
- Hospitalisation for non-diabetes related reasons
- Hospitalisation due to road traffic accident

6.5 Pregnancy

If a patient becomes pregnant whilst involved in this study, it is not considered to be an SAE or an AE. If a patient becomes pregnant, this must be recorded on the CRF, including details of gestation and outcome.

6.6 Insurance

Sponsor's public liability will be provided by the University of Exeter for the design and management of the study. NHS indemnity will apply for the conduct of the study at the site.

7. Data Handling & Record Keeping

7.1 Confidentiality

The CI is responsible for ensuring that participant anonymity is protected and maintained, ensuring that their identities are protected from any unauthorised parties. The CI is the 'Custodian' of the data. All information related to study participants will be kept confidential and managed in accordance with the UK GDPR and Data Protection Act 2018, NHS Caldicott Guardian, UK Policy Framework for Health & Social Care, and Research Ethics Committee Approval.

A unique Study ID will be allocated to each participant. All study data and samples will be pseudo-anonymised and stored under the Study ID on a secure password-protected study database managed by the central coordinating team. Personal identifiable data will only be accessible to personnel with training in data protection who require this information to perform their study role. Personal data to be collected will include, name, date of birth, NHS or CHI number, gender, contact details and preferences. Identifiable information will be stored on a separate, secure password-protected database held on an NHS server to enable the research team to undertake the study. Only those members of the research team whose role requires access to personal identifiers will have access. The samples are processed by registered healthcare scientists and are afforded the stringent information governance as given to all clinical samples.

All paper copies of study data will be stored under ID number and kept in locked, access-controlled offices within the research facilities; research data will be held separately to identifiable information. Researchers involved in data analysis will not have access to personal identifiable data, only the anonymised research data. No identifiable data will be included in research publications or progress reports.

Any participant information required to be sent to a third party will adhere to these pseudo-anonymised parameters, this includes the participant's date of birth, gender as well as unique study ID. No participant identifiable data will be sent outside the EU.

Anonymised clinical study data will be shared on vivli.org or similar funder and sponsor approved participant-level data repository.

7.2 Case Report Form (data collection form)

The relevant visit Case Report Form (CRF) will capture all the information required to ensure that all the documented statistical information thus dictated in the protocol is captured and documented at each visit. This also serves to monitor patient eligibility and safety at Sponsor level.

8. Study Documents

- Signed protocol and any subsequent amendments
- Sponsor Monitoring Plan and subsequent reports
- Data Management Plan
- Current/Superseded Patient Information Sheets (as applicable)
- Current/Superseded Consent Forms (as applicable)
- Indemnity documentation from Sponsor
- Conditional/Final R&D Approval
- Signed site agreement
- Ethics/HRA submissions/approvals/correspondence
- CVs of CI/PI and site staff
- UK regulations (GCP) course certificate of CI/PI

- Delegation Log
- Staff Training Log
- Recruitment Log
- Monitoring Visit Log
- Breach of Protocol Log
- Case Report Forms (Data Collection Forms)
- Correspondence relating to the study

9. Record Retention and Archiving

During the course of research, all records are the responsibility of the Chief Investigator / Principal Investigator and must be kept in secure conditions. When the research study is complete, it is a requirement of the UK Policy Framework for Health & Social Care and Sponsor Trust Policy that the records are kept for a further 15 years. At the end of the study, anonymised data will be included in the study's final dataset and stored indefinitely in a sponsor/funder approved data repository. Personal data will be stored where consent is given by the participant to be contacted for follow-up on their future health status (stored for 15 years after the study ends), and/or about participating in future studies (stored indefinitely or until the participant asks for their personal data to be deleted). Where consent is given by the participant, their remaining samples and anonymised data from the project will be gifted to the Peninsula Research Bank (an approved tissue bank, REC ref 19/SW/1059) in Exeter to be used for future research.

At the end of the study, it will be archived by the CI at the University of Exeter.

10. Statistical Considerations

10.1 Feasibility

The primary analysis will be assessment of the correlation between hypoglycaemia questionnaire data (Clarke and Gold scores, including total number of symptomatic and asymptomatic episodes of hypoglycaemia in the month prior to assessment), CGM glucose variability and hypoglycaemia metrics [27] against peak post MM or AST plasma glucagon. We expect that glucose variability metrics will be a more powerful comparator than episodes of hypoglycaemia due to a likely small number of clinically significant hypo episodes during the CGM period. We will test association of glucagon adjusted for other clinical variables known already to predict hypoglycaemia. We expect that stimulated glucagon will have a closer relationship with hypoglycaemia than fasted glucagon, as per our original pilot data, and will be able to confirm this in this study. We will compare MM stimulated glucagon to AST stimulated glucagon.

10.2 Statistical analysis

For a sample size of 75, it should be possible to detect a correlation between stimulated glucagon and hypoglycaemia of r=0.3, with 95% confidence intervals 0.08 to 0.49. We will also correlate MM-induced increases in plasma glucagon to plasma co-peptin.
Test order at Visits 1 and 2 will be randomised and assigned at time of recruitment via a computer-generated list stored in the back end of the study database, blinded to the recruitment team.

11. Compliance

The CI will ensure that the study is conducted in compliance with the principles of the Declaration of Helsinki (2013), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the UK Policy Framework for Health & Social Care and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006, 2008 and 2009 and any future relevant or replacement EU Regulations, Trust and R&D Office policies and procedures and any subsequent amendments.

12. Clinical Governance Issues

12.1 Ethical Considerations

This protocol and any subsequent amendments, along with any accompanying material provided to the patient, will be submitted by the Chief Investigator to an independent Research Ethics Committee (REC) and Health Research Authority (HRA). Written REC and HRA approvals will be obtained and subsequently submitted to the Sponsor R&D to obtain Sponsor approval prior to commencing the study. Local confirmation of capacity and capability will be in place before the sponsor initiates the 'green light' for research activity to commence.
To minimise risk, we have: i) back up medical doctor cover on site, and ii) an experienced diabetes specialist nurse (with training/experience of hypoglycaemia and giving insulin correction doses) recruiting to and running the study.
Venepuncture/Cannulation in each test may result in slight bruising and discomfort but will be minimised by the procedure being undertaken by researchers experienced in cannulation.

Patient burden:
Mixed Meal Tolerance Test (MMTT) and Arginine Stimulation Test (AST)

- Fasting prior to the stimulation tests can increase the risk of hypoglycaemia. The Mixed Meal Tolerance Test (MMTT) and Arginine Stimulation Test (AST) carry a risk of hyperglycaemia (from having a meal without their usual mealtime insulin). Participants will be closely monitored during and after their visit by the diabetes specialist research team. Appropriate advice/support will be given if required.

Continuous Glucose Monitoring (CGM)

- The fitting of the CGM sensor may result in slight discomfort which will be minimised by the procedure being carried out by researchers experienced in their use.
- Appropriate advice & support will be provided.
- Participants are under no obligation to take part in CGM.

13. Quality Control and Quality Assurance

13.1 Monitoring

Monitoring of this study will ensure compliance with Good Clinical Practice. The Investigator(s) will permit monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (e.g. participants' case report forms, consent forms etc). Periodic remote monitoring will be carried out by the CI's study team and the Sponsor. Consent will be taken to permit access to data by external staff responsible for monitoring and auditing the study as a requirement of participating in the study. Remote monitoring of recruitment activities will be carried out periodically by the study management team (on behalf of the CI & Sponsor) by exploring data, consent forms, delegation log & recruitment log in accordance with the study SOPs/work instructions.

13.2 Audit and Inspection

Auditing Definition: "A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, Sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s)."

A study may be identified for audit by any method listed below:
1. A project may be identified via the risk assessment process.
2. An individual investigator or department may request an audit.
3. A project may be identified via an allegation of research misconduct or fraud or a suspected breach of regulations.
4. Projects may be selected at random. The Department of Health states that Trusts should be auditing a minimum of 10% of all research projects.
5. Projects may be randomly selected for audit by an external organisation (e.g. MHRA).

Internal audits will be conducted by a Sponsor's representative.

13.3 Serious Breaches in GCP or the Trial Protocol

The Sponsor of the clinical trial is responsible for notifying the licensing authority in writing of any serious breach of:

- the conditions and principles of GCP in connection with that trial; or
- the protocol relating to the trial, as amended from time to time in accordance with regulations, within 7 days of becoming aware of that breach.

For the purposes of this regulation, a 'serious breach' is a breach that is likely to effect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial.

The CI is responsible for reporting any serious breaches to the Sponsor (R&D) within 24 hours.

13.4 Non-Compliance

Defined as a noted systematic lack of both the CI and the study staff adhering to SOPs/work instructions/protocol/ ICH-GCP and UK regulations, which leads to prolonged collection of deviations, breaches or suspected fraud.

These non-compliances may be captured from a variety of different sources including monitoring visits, CRFs, communications and updates. The Sponsor will maintain a log of the non-compliances to ascertain if there are any trends developing which need to be escalated. The Sponsor will assess the non-compliances and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the R&D will agree an appropriate action, including an on-site audit.

14. Study Committees

The Study Management Committees will be defined as:

- Study Management Group (SMG)
- Study Steering Committee (SSC)

15. Public and Patient Involvement

The study team has access to the user representative group of the NIHR Exeter Clinical Research Facility (Exeter CRF) and a Diabetes PPI group. In keeping with the NHS Patient Carer and Public Involvement (PCPI) strategy, the Exeter CRF invites user representatives to contribute to the development of various projects within its portfolio. These individuals have agreed to maintain contact and regular meetings have been established at which researchers discuss the development of current projects within the Exeter CRF.

The Exeter CRF user group and Diabetes PPI group have been actively involved in the study wording of the patient-facing documents for this study.

Study progress will be overseen by a steering group which will include external lay and patient members.

16. Publication Policy

Results will be written up and submitted for publication in a peer-reviewed journal(s). Abstracts will be submitted to national and international conferences. Results will be presented to clinical colleagues at regular in-house meetings. Written information in the form of a letter/newsletter outlining the key findings of the study will be sent to all participants and will be uploaded to the study website. It may be possible to organise in-person/online events to present the study findings to participants.

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18. Appendices

18.1 Appendix 1 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
N/A – response to original submission	2	04/11/2022	Richard Oram & Michelle Hudson	• Inserted new University of Exeter Sponsor logo • Provided clarity regarding sample destruction and data storage.