Non-CTIMP Study Protocol

Aniracetam for the Prevention of Hypoglycaemia in Type 1 Diabetes

An exploratory, *in vivo*, physiological study

Co-Sponsors	The University of Edinburgh and/or Lothian Health Board ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Protocol Authors	**Dr Victoria Tyndall** Dr Nicola Baillie Professor Shareen Forbes
Funder	Helmsley Charitable Trust Foundation
Chief Investigator	Professor Shareen Forbes
Trial Management Committee	Prof Robert Semple (Diabetes and Endocrinology) Dr Alixe Kilgour (Geriatric Medicine) Dr Caelan Taggart (Cardiology) Ms Tara J Wilson (Patient Representative for Type 1 Diabetes)
Data Monitoring Committee	Prof. James Dear (Toxicology) Dr Christopher Box (Rheumatology) Dr Rose Penfold (Geriatric Medicine)
Sponsor Number	AC 24198
REC Number	25/ES/0079
R&D Number	2025/0235
Project Registration	ISRCTN 9590 1685
IRAS ID	349 276
Version Number and Date	Version **6.0 last updated** 08Sep2026

TABLE OF CONTENTS

TABLE OF CONTENTS 2
1. ABBREVIATIONS 5
2. INTRODUCTION 7
2.1 GLUCAGON AND THE COUNTERREGULATORY RESPONSE (CRR) 7
2.2 STUDY RATIONALE 8
2.3 PHARMACOLOGY OF ANIRACETAM 9
1.1.1 Aniracetam pharmacology in *ex-vivo* human experiments 9
1.1.2 Aniracetam pharmacology in *in-vivo* animal experiments 10
1.1.3 Aniracetam pharmacology in clinical human studies 10
1.1.4 Choosing the Aniracetam dose for our study 10
3. STUDY OBJECTIVES AND ENDPOINTS 11
3.1 OBJECTIVES 11
3.2 ENDPOINTS 11
2.2.1. Primary Endpoint 11
2.2.2. Secondary Endpoints 12
4. STUDY DESIGN 12
5. STUDY MEDICATIONS 13
5.1 ANIRACETAM 13
5.1.1. Study medication identification 13
5.1.2. Study medication manufacturer 13
5.1.3. Marketing Authorisation holder 13
5.1.4. Labelling and Packaging 13
5.1.5. Shipping and import 13
5.1.6. Regulatory Release to Site 14
5.1.7. Destruction of Products 14
5.1.8. Summary of Product Characteristics Booklet or Investigator's Brochure 14
5.2 PLACEBO 14
5.2.1. Labelling and Packaging 14
5.2.2. Storage 15
5.3 Dosing Regime 15
6. Study Population 15
6.1 NUMBER OF PARTICIPANTS 15
6.2 INCLUSION CRITERIA 15
6.3 EXCLUSION CRITERIA 16
6.4 CO-ENROLMENT 16
6.5 PARTICIPANT SELECTION, ENROLMENT AND WITHDRAWAL 16

6.5.1. IDENTIFYING PARTICIPANTS 16
6.5.2. CONSENTING PARTICIPANTS 17
6.5.3. WITHDRAWAL OF STUDY PARTICIPANTS 17
6.6 PATIENT AND PUBLIC INVOLVEMENT 18
7. STUDY ASSESSMENTS 19
7.1 VISIT 1: ENROLMENT AND ELIGIBILITY ASSESSMENT 20
7.1.1. Arginine Simulation Testing 20
7.2 RANDOMISATION 21
7.3 STUDY VISITS 2 AND 3: HYPOGLYCAEMIC CLAMPS 22
7.3.1. Participant preparation for clamp 22
7.3.2. Postponing a hypoglycaemic clamp 22
7.3.3. A hypoglycaemic clamp 23
7.3.4. After a clamp – restoration 25
7.3.5. Blinding of Aniracetam vs Placebo 27
7.3.6. Discharge following clamp experiments and participant compensation 27
7.3.7. Follow up assessments 27
7.3.8. Storage and analysis of samples 27
7.3.9. Data collection 27
7.3.10. Source Data Documentation 28
7.4 Case Report Forms 28
8. DATA MANAGEMENT 28
8.1 PERSONAL DATA 28
8.2 DATA INFORMATION FLOW 29
8.3 DATA STORAGE 29
8.4 DATA RETENTION AND DISPOSAL 29
8.5 EXTERNAL TRANSFER OF DATA 30
8.6 DATA CONTROLLER 30
8.7 DATA BREACHES 30
8.8 DATA MONITORING COMMITTEE 30
9. STATISTICS AND DATA ANALYSIS 30
9.1 SAMPLE SIZE CALCULATION 30
9.2 PROPOSED ANALYSES 31
9.2.1. Summary measures to be reported: 31
9.2.2. Method of analysis: 31
9.2.3. Plans for handling missing, unused, and spurious data; non-compliers and withdrawals: 31
9.2.4. Plans for pre-defined subgroup analyses: 31
9.2.5. Statement regarding use of intention to treat (ITT) analysis: 32

10. ADVERSE EVENTS 32
10.1 ADVERSE EVENT REPORTING 32
10.2 SERIOUS ADVERSE EVENT REPORTING 33
10.3 SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION REPORTING 33
10.4 UNBLINDING 33
10.5 URGENT SAFETY MEASURES (USM) 34
10.6 SAFETY EVENT FOLLOW UP 34
11. OVERSIGHT ARRANGEMENTS 34
11.1 INSPECTION OF RECORDS 34
11.2 STUDY MONITORING AND AUDIT 34
11.3 STUDY STEERING COMMITTEE 35
11.4 DATA MONITORING COMMITTEE 35
12. GOOD CLINICAL PRACTICE 35
12.1 ETHICAL CONDUCT 35
12.2 INVESTIGATOR RESPONSIBILITIES 35
12.2.1. Informed Consent 35
12.2.2. Study site staff 36
12.2.3. Data recording 36
12.2.4. Investigator documentation 36
12.2.5. GCP Training 36
12.2.6. Data Protection Training 36
12.2.7. Information Security Training 37
12.2.8. Confidentiality 37
12.2.9. Data Protection 37
13. STUDY CONDUCT RESPONSIBILITIES 37
13.1 PROTOCOL MODIFICATIONS (PREVIOUSLY KNOWN AS AMENDMENTS) 37
13.2 MANAGEMENT OF PROTOCOL NON COMPLIANCE 38
13.2.1. Protocol Waivers 38
13.2.2. Management of Deviations and Violations 38
13.3 SERIOUS BREACH REQUIREMENTS 38
13.4 STUDY RECORD RETENTION 38
13.5 END OF STUDY 38
13.6 CONTINUATION OF TREATMENT FOLLOWING THE END OF STUDY 39
13.7 INSURANCE AND INDEMNITY 39
13.8 AUTHORSHIP POLICY 39
14. REFERENCES 40

1. ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research and Development
AE/AR	Adverse Event / Adverse Reaction
APH1D	Aniracetam for the Prevention of Hypoglycaemia in Type 1 Diabetes
ArST	Arginine Stimulation Test
CGM	Continuous Glucose Monitoring
Ca	Calcium
CF	Consent Form
CI	Chief Investigator
CRF	Clinical Research Facility
DMC	Data Monitoring Committee
EMA	European Medicines Agency
ERI	Edinburgh Royal Infirmary
FDA	United States Food and Drug Administration
g	Grams
GCP	Good Clinical Practice
GP	General Practitioner
ICH	International Conference on Harmonisation
IP	Intraperitoneally
ITT	Intention To Treat
LD	Lethal Dose
mg	Milligram
uM	Micromole
nM	Nanomole
PI	Principal Investigator
pmol	Picomole
QA	Quality Assurance
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SH	Severe Hypoglycaemia

SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
SOP	Standard Operating Procedure
TBR	Time Below Range
TIR	Time In Range
T1D	Type 1 Diabetes Mellitus

2. INTRODUCTION

Type 1 Diabetes (T1D) is an autoimmune condition which most commonly develops in childhood, but can occur at any age[1]. It is characterised by insulin deficiency caused by autoimmune destruction of the insulin-producing β-cells of pancreatic islets. In health, glucagon and insulin released from α- and β-cells respectively within the pancreatic islet act in a delicate balance to regulate glucose levels. Insulin drives glucose into cells so that it can be used as energy, while glucagon drives release of glucose stores from the liver to prevent hypoglycaemia. In T1D, this balance is lost, and people with T1D must self-administer and adjust their insulin doses multiple times per day in order to allow their body to properly store and metabolise glucose as energy.

Hypoglycaemia is therefore an understandably common consequence of insulin therapy[2]. Despite advances in glucose monitoring as well as insulin formulation and delivery, hypoglycaemia remains a significant clinical challenge for patients and physicians alike[3]. Recurrent hypoglycaemia can have a devastating impact on patients and their loved ones as in the acute setting, severe hypoglycaemia (SH) is a cause of permanent brain injury, seizures, and even death[1], while longer term it predisposes to accelerated cognitive decline[4].

Current treatment strategies for hypoglycaemia involve glucose monitoring and administration of carbohydrates or glucagon. However, these approaches may not always be effective in preventing or resolving hypoglycemic episodes. Additionally, they are not preventative, and fail to address underlying metabolic imbalances that contribute to hypoglycaemia susceptibility. There is a need for novel and proactive therapeutic interventions which can complement existing treatments for hypoglycaemia in individuals with T1D. Research using extracted tissue from people with T1D and animal models has shown that the mechanism of glucagon release in T1D is switched off but may potentially be reactivated using modulators of the AMPA/Kainate cell signalling pathway. Aniracetem has been selected as the positive allosteric modulator in this study.

2.1 GLUCAGON AND THE COUNTERREGULATORY RESPONSE (CRR)

Glucagon is produced from α-cells in the islets of the pancreas and is a sensitive regulator of glucose homoeostasis *in vivo* in humans. It is a critical hormone in the counter-regulatory response (CRR) to hypoglycaemia[5-7]. These *in vivo* observations have been confirmed *ex vivo*: α-cells within living pancreas slices from donors with T1D do not mount an adequate glucagon response to hypoglycaemia and are one useful model to study such metabolic defects in T1D[8]. Regulation of glucagon secretion by the pancreatic α-cell remains incompletely understood, although electrophysiological properties of the α-cell and glutamate receptor signalling are implicated[9-11]. Recent research suggests that α-cells potentiate their own secretory activity via an ionotropic glutamate receptor autocrine feedback mechanism[11]. In the non-diabetic state, a low glucose leads to the release of glutamate that in turn enhances glucagon secretion with restoration of blood glucose levels.

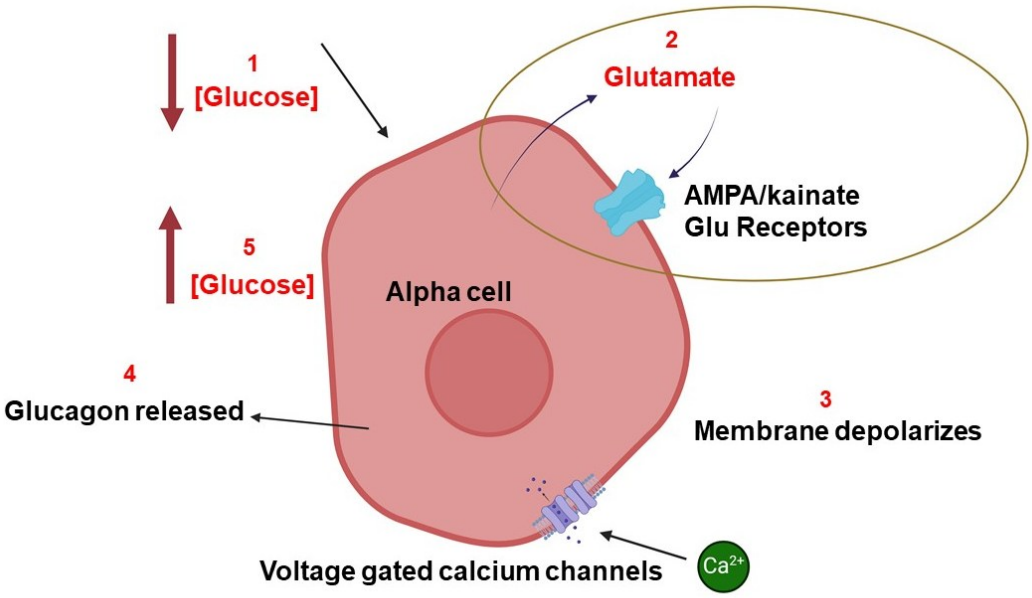


Figure 1: Glutamate receptor autocrine feedback signalling in the pancreatic α-cell in normal glucose tolerance. Glutamate elevates Glucagon responses as follows: (1) Lower glucose levels are detected resulting in (2) release of glutamate from the α-cell which acts on ionotropic glutamate receptor iGluRs of the AMPA/kainate type, with (3) membrane depolarization, opening of voltage-gated calcium (Ca^{2+})channels and an increase in cytoplasmic free Ca^{2+} concentration with (4) enhanced glucagon release and elevation/resoration of glucose concentrations (5).

2.2 STUDY RATIONALE

A person without T1D unaccustomed to hypoglycaemia will typically exhibit signs and symptoms such as palpitations, shakiness, sweating and even confusion if their blood glucose is lowered to 3.9 mmol/l or below. These symptoms reflect a CRR, driven largely by glucagon and other hormones (such as adrenaline and growth hormone) which has evolved to increase glucose levels in response to hypoglycaemia.

Almost all people with T1D develop a specific defect in α-cell glucagon secretion by 5-years disease duration[6,9]. This defective CRR of glucagon to hypoglycaemia, markedly increases the risk of SH[5]. Additionally, previous studies have shown that age, duration of T1D as well as recurrent and prolonged episodes of hypoglycaemia over time all contribute to impaired awareness of hypoglycaemia and risk of SH.

Hypoglycaemic clamp studies, such as the one proposed here, were initially developed in the 1980s and are considered the gold-standard for investigating pancreatic islet and cell function in both health and disease[12]. The subject receives an infusion of insulin with dextrose via a dual-lumen cannula. The infusion rates are adjusted based on body measurements and point-of-care glucose measurements in order to physiologically "clamp" their plasma glucose at a particular level[13]. During a hypoglycaemic phase of the clamp, investigations such as blood sampling, neurocognitive testing and symptom scoring can be undertaken safely in a controlled environment[12,13]. It is important to note that This study does not aim to establish therapeutic efficacy for marketing authorisation, but to characterise a physiological mechanism.

Our collaborators have shown that isolated human islets exposed to 1 nM of AMPK modulators, can increase glucagon secretion by between two and four-fold [9]. A similar response has been demonstrated in rodent T1D models[9,11]

We therefore plan to use clamp experiments to determine whether the mechanism of glucagon release can be reactivated *in-vivo* in people with T1D using AMPA/kainate modulation [17,23,24].

2.3 PHARMACOLOGY OF ANIRACETAM

Aniracetam (Ro 13-5057) is a member of a group of compounds that have in common a 2-pyrrolidone ring, which in turn forms a subgroup of a pharmacological class of compounds referred to as nootropics[14]. It is a positive allosteric modulator of the AMPA/kainate glutamate receptor[15]. Such allosteric modulators offer a way to fine-tune receptor activity with greater specificity and potentially fewer side effects, enhancing the receptors response to glutamate as compared to direct agonists. Aniracetam is currently used to treat cognitive decline associated with dementia and Alzheimer's disease, with some evidence of enhanced cognition in these groups[14,15].

1.1.1 Aniracetam pharmacology in *ex-vivo* human experiments

Aniracetam has not been given to people with T1D and it is unknown whether it increases their glucagon response to hypoglycaemia. Pre-clinical data generated by Caicedo *et al* is encouraging: Aniracetam (range - 1-100nM) restored glucagon secretion *in vitro* in living pancreas slices from donors with T1D [8]. Isolated human islets exposed to 1nM of aniracetam increased glucagon secretion by two-fold with the glucose concentration held constant at 5.5 mM (Figure 2; Caicedo laboratory[8]).

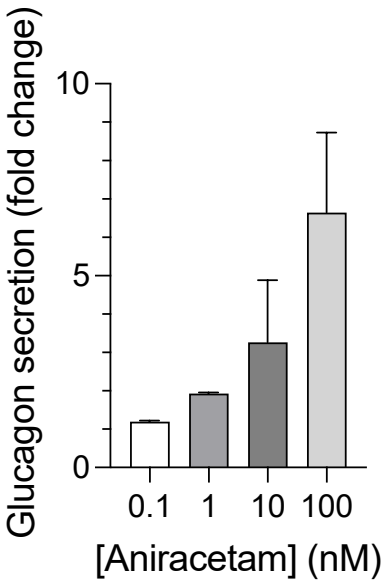


Figure 2: Aniracetam increases glucagon release from isolated human islets in a dose-response relationship[8]: [Glucose] was constant at 5.5 mM (n=3)

Furthermore, Aniracetam at a dose of 30mg/kg administered intraperitoneally (IP) in non-obese diabetic mice, restored glucagon secretion and glucose counter regulation *in vivo* in T1D mouse models[8,10]. Circulating levels of Aniracetam were not measured in these pre-clinical models, and the value of exploring these observations further in mice is limited due to inherent differences between humans and mice.

1.1.2 Aniracetam pharmacology in *in-vivo* animal experiments

To conduct a feasible study in participants with T1D, dose-effective circulating concentrations of Aniracetam must be attained. An effective dose can be extrapolated by considering what we already know about the pharmacokinetics and pharmacodynamics of Aniracetam in previous clinical and animal experiments.

In animals, Aniracetam is rapidly and complete absorbed by the GI tract, with 98% of the compound excreted in the urine after hepatic metabolism and peak plasma levels reached between 5-30 minutes after ingestion. In mice, intraperitoneal Aniracetam is typically dosed at 30 mg/kg[16]. Using the FDA's body surface area–based conversion (Km_mouse = 3; Km_human ≈ 37) this corresponds to approximately 2.44 mg/kg in a human[17,18]. For a 70 kg person this would be 170 mg. Although a much lower dose than a 1500 mg tablet, the routes of administration (and therefore first-pass metabolism) are different.

1.1.3 Aniracetam pharmacology in clinical human studies

In humans, 80% of circulating Aniracetam is excreted in the urine within the first 24 hours following administration and the compound is out of the system after 150 minutes (five half-lives) with no evidence of accumulation[19] Aniracetam has several metabolites (2-pyrrolidinone, 5-hydroxy-2-pyrrolidinone, succinimide, anisic acid and anisoyl-GABA), the majority of which is anisoyl-GABA. Each of these metabolites demonstrate pharmacological activity similar to the unchanged product and principal metabolites are detected in the blood 6-12 hours following ingestion[20].

The maximum dose of Aniracetam which is routinely administered to human patients is a single dose of 1500 mg. It is rapidly absorbed from the gastrointestinal tract in both humans and animals, and in humans 66% of the circulating compound bound to plasma proteins, leaving 34% of the compound freely bioavailable. *In vivo* human studies have documented a total (free + bound) peak concentration of 14 ug/L (which is equivalent to 64 nM accounting for the molecular weight of Aniracetam which is 219.2365 g/mol) approximately 30 minutes after ingestion of 1200mg Aniracetam[15,21,22]. This is equivalent to a free bioavailable concentration of approximately 22 nM[19] .

1.1.4 Choosing the Aniracetam dose for our study

Based on the above, we can infer that circulating free Aniracetam levels in humans reach ~ 22 nM after an oral dose of 1200 mg. We can extrapolate the data in Figure 2 and use it to surmise that a 1200mg dose of Aniracetam should increase glucagon secretion approximately four-fold (see Figure 3). To place this in context, a lowering in glucose concentration from 17 mM to 1 mM elicits a 2-fold increase in glucagon and potassium-chloride depolarisation (which is a very strong experimental stimulus to elicit glucagon release *ex-vivo*) elicits a five-fold increase in glucagon secretion.

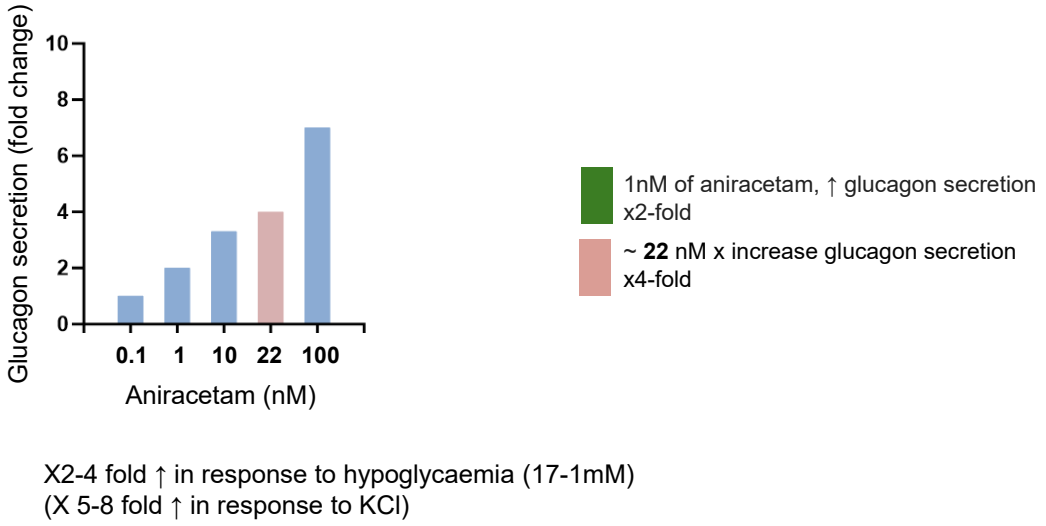


Figure 3: Extrapolation as to expected fold increase in glucagon secretion if circulating free levels of Aniracetam of 22mM are chieved (adapted from Caicedo lab data)[8].

If we consider this response in the context of what is known (personal communication from A Caicedo) this is around a four-fold increase in glucagon secretion from baseline. This is similar to the sort of response seen in normal glucose tolerant individuals after exposure to hypoglycaemia. Hence, we can theorise that a dose of 1200-1500mg Aniracetam given to humans with T1D should restore their counter-regulatory glucagon response. We therefore plan an exploratory physiological study in participants with T1D to test this theory. A new therapeutic modulator of hypoglycaemic response has the potential to reduce both the severity and frequency of hypoglycaemia in people with T1D. Our study will be the first controlled human investigation of this mechanism and we will use a standard 1500mg dose.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 OBJECTIVES

Our primary objective is to determine whether positive allosteric modulation of the α-cell AMPA/kainate receptors, can modulate glucagon release during hypoglycaemia in people with T1D. This will assist us in further characterising the physiology of α-cell glucagon secretion during insulin-induced hypoglycaemia in people with T1D.

3.2 ENDPOINTS

2.2.1. Primary Endpoint

Our primary endpoint will be the change in plasma glucagon concentration from euglycemia to hypoglycaemia during and after hyperinsulinaemic clamp, when the AMPA/kainate pathway is modulated.

2.2.2. Secondary Endpoints

As secondary endpoints, since glucagon can be challenging to assay, we will also assess two indirect measures of glucagon secretion : (a) time to reach euglycaemia after hypoglycaemia and (b) the amount of dextrose required to attain euglycaemia following hypoglycaemia. We will also assess the response of other hormones involved in hypoglycaemia counter-regulation; namely cortisol, adrenaline, noradrenaline and growth hormone. In addition, at each visit we will profile the reproductive hormones and reproductive status of each participant as this is known to impact the CRR (i.e. different responses have been observed in males and females) [23-25].

4. STUDY DESIGN

This is a double blinded, 1:1, crossover, placebo controlled, exploratory physiological study undertaken on a single site as shown in Figure 4 below.

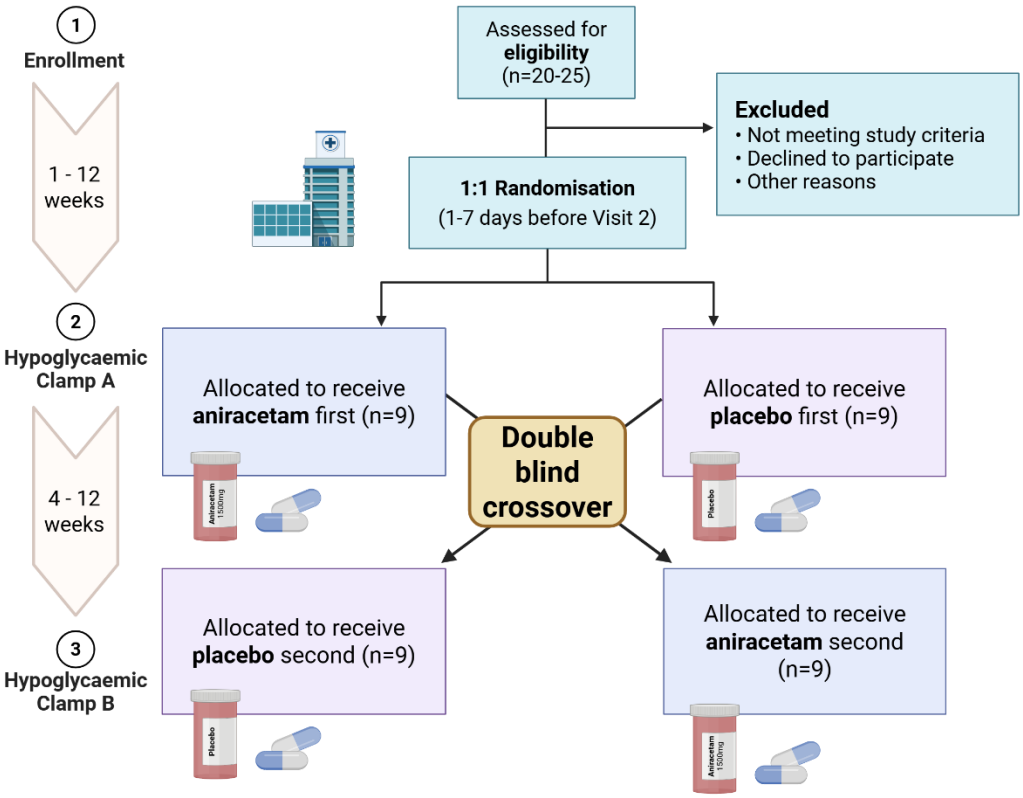


Figure 4: Study schedule/outline: *After a satisfactory screening/enrolment visit 1 and provided they satisfy the study criteria, participants will attend for two further visits to undergo hyperinsulinaemic-hypoglycaemic clamp. Between one and seven days prior to visit 2, they will be randomised by pharmacy to receive either Aniracetam or placebo on the day of each clamp. Neither investigators nor participants will know whether the two tablets received are placebo or Aniracetam.*

5. STUDY MEDICATIONS

5.1 ANIRACETAM

5.1.1. Study medication identification

Aniracetam

5.1.2. Study medication manufacturer

Lavipharm. 12 Agias Marinas Street. PO Box 59 GR-19002 Peania Attica, Greece

5.1.3. Marketing Authorisation holder

Lavipharm

5.1.4. Labelling and Packaging

Lavipharm have manufactured one box of 60 tablets of 750mg aniracetam (Memodrin ™) and one box of 60 tablets of matched placebo which are pictured in Figure 5.

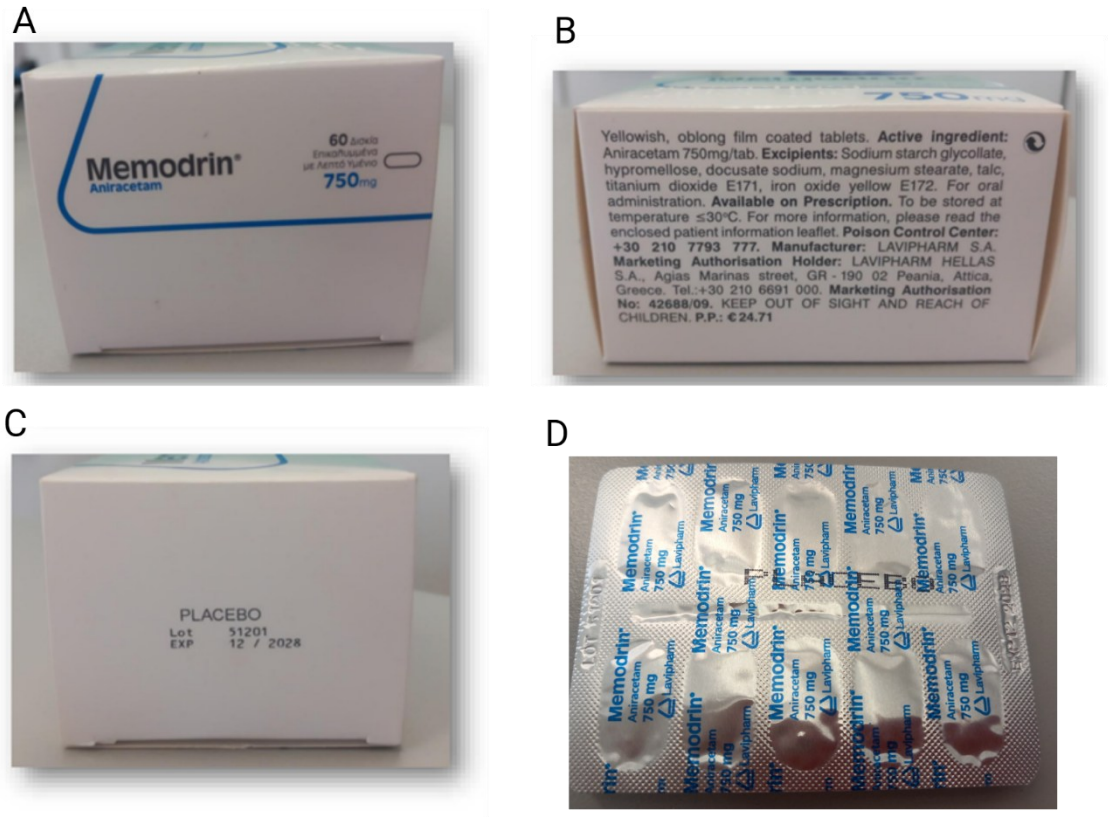


Figure 5: Aniracetam (A&B) and Placebo (B&D) packaging: Two boxes are being shipped of 60 tablets each, one containing aniracetam (A) and the other a matched placebo (C). The text description on the box is in English (B) and the blister packs are also labelled if they are placebo (D).

5.1.5. Shipping and import

Both Aniracetam and Placebo will have a three year expiry and will be packaged and labelled in English (Figure 5). In advance of the study starting, our pharmacy QA department have:

- An English translation of the patient information leaflet and the product labels
- Photographs of the primary packaging (blisters) and secondary packaging (cartons)
- A certificate of Analysis and Certificate of Conformity for a current batch of the active and placebo tablets. When pharmacy receive any shipments we will require the CoC and CoA for each batch received.

PNR Pharma will be responsible for importation of Aniracetam and placebo into the UK. The address for PNR Pharma is Consulting Ltd, Unit 5 And 6 Lime Avenue, Ebbw Vale, Blaenau Gwent, United Kingdom, NP23 6GL.

As per the MHRA, import will occur under PNR Pharma's "Specials" licence and their team will complete an Import Submission to the MHRA (this takes approximately 28 days). Import from Greece to the UK by PNR Pharma will involve an Importer of Record to oversee the process. We have contracted Fiscal IOR to do this. Once the MHRA have approved the import, PNR Pharma will then complete their own quality checks in accordance with the Quality Technical Agreement and provide a quality statement before shipping to our clinical pharmacy at the ERI.

The clinical pharmacy team can then (under hospital exemption) add additional information at the point of dispensing if required (see 5.1.4).

Products will be stored in the NHS Pharmacy, ERI, at room temperature (below 30°C). No specific temperature monitoring is required in transit.

5.1.6. Regulatory Release to Site

Lavipharm will QP release the products to PNR Pharma for shipment. PNR Pharma will further provide a quality statement to confirm the correct packs were received in good order and at the correct temperature from Lavipharm, and stored appropriately in PNR's warehouse before shipping the product on the NHS Pharmacy, ERI.

5.1.7. Destruction of Products

Any remaining clinical study medication will be destroyed according to the NHS Pharmacy's destruction SOP. This will be retained in the Pharmacy's site file.

5.1.8. Summary of Product Characteristics Booklet or Investigator's Brochure

The Aniracetam Summary of Product Characteristics (SmPC) is provided in a separate document with a cover sheet and signature page (signed and verified by the CI and Co-Sponsors) and is filed in the TMF. Please refer to Aniracetam Investigator's Brochure.

5.2 PLACEBO

Placebo will be prepared by Lavipharm in the same manufacturing site as the Aniracetam and imported in the same way at the same time as the active Aniracetam by PNR Pharma. Lavipharm will prepare a visually identical placebo, with exact constituents, minus the Aniracetam component. The placebo will therefore not require additional stability testing.

5.2.1. Labelling and Packaging

Medication labels will be in English They will include storage conditions for the product, but no information about a patient until the products are dispensed by pharmacy.

Dispensing will take the form of a label applied to each HDPE bottle of two-tablets (they will pop the active/placebo tablets out of the blister into a bottle the day before dispensing).

A blinded label will then be applied to the bottle which will include the product as "aniracetam/placebo", quantity, strength and formulation, the participant details (including randomisation number and study ID), sponsor details, investigator name, and directions for use.

5.2.2. Storage

Aniracetam and placebo will be stored in NHS Pharmacy, ERI, at room temperature (below 30°C). No specific monitoring is required but pharmacy temperature logs will be checked at monitoring visits as a precautionary measure.

5.3 Dosing Regime

Pharmacy at the ERI will be sent:

- 1 box of 60 film coated tablets containing API (Aniracetam 750mg) in blister strips
- 1 box of 60 film coated tablets identical placebo to match, in blister strips.

Clinical pharmacy will hold a randomisation/ allocation list created by our lead statistician at University of Edinburgh. Following randomisation, pharmacy will prepare a prescription for each participant which they will check for completeness prior to dispensing against a randomisation list. Pharmacy will dispense 2 tablets of aniracetam or placebo to each patient in an HDPE bottle the working day prior to administration, double checked against the randomisation list prepared by the statistician (see Section 7.2). No stability testing in HDPE bottles is required as the clinical conditions in which the products are kept will not be changing. The working day prior to administration, a prescription to be signed by the investigator and the aniracetam/placebo with it's blinded label attached will be delivered to the clinical research facility (CRF) at the ERI. The investigator will sign the blinded prescription and the aniracetam/placebo will be administered in accordance with the study protocol.

6. Study Population

6.1 NUMBER OF PARTICIPANTS

We will consent up to 25 participants to attend an enrolment and screening visit with an aim to recruit 18 participants to undergo clamp experiments. . They will be recruited over a 12 month period. We aim to recruit 9 male and 9 female participants. We expect that up to 20% of participants recruited will not be eligible for inclusion following the investigations undertaken at visit 1 or will decide not to proceed further with the study. We are aiming to recruit more participants at visit 1 to account for this.

6.2 INCLUSION CRITERIA

1. Participants with T1D for 5 years or more.
2. Age 21-65 years.
3. Participants with T1D with C-peptide levels less than 50pmol/l

6.3 EXCLUSION CRITERIA

1. Undergoing active treatment for proliferative diabetic retinopathy or diabetic maculopathy
2. History of Diabetic ketoacidosis in the preceding six months.
3. Severe hypoglycaemic episode requiring external assistance in the preceding six months.
4. Time below range on continuous glucose monitoring (CGM) of more than four percent for the two weeks preceding visit 1.
5. Cardiovascular disease including previous myocardial infarction (MI), ischaemic heart disease, heart failure, stroke or transient ischaemic attack (TIA), peripheral vascular disease and untreated (or uncontrolled) hypertension.
6. Chronic kidney disease (CKD) stages 3 to 5
7. Medical history of Haemophilia, Cystic Fibrosis, pancreatic disease or complete pancreatectomy, ischaemic heart disease, cardiac arrhythmia, phenylketonuria epilepsy or hypoglycaemia induced seizure
8. History of severe reaction or allergy to adhesive necessary to this study
9. Unable or unwilling to adhere to study timetable
10. Unable or unwilling to give informed consent
11. Pregnancy or planning pregnancy
12. Concurrent use of any non-insulin glucose-lowering agent which could affect the CRR, predispose to DKA or affect insulin requirements (including GLP-1 and GLP-1/GIP agonists (Ozempic, Wegovy, Mounjaro), Symlin, DPP-4 inhibitors, SGLT-2 inhibitors, sulfonylureas).
13. Untreated hypothyroidism
14. Concurrent use of medication that may affect blood glucose and cannot be reasonably omitted, such as SSRIs
15. Inability to understand or speak English. Time is of the essence during the clamp procedure, there is no time for translation
16. A condition, which in the opinion of the investigator, would put the patient or study at risk

6.4 CO-ENROLMENT

Study staff will discuss with participants on a case-by-case basis if they are planning on enrolling in another interventional study. The participant and study staff must consider the burden on the participant of being enrolled in more than one study. In addition, any other study that a participant enrolled in would need to have no impact on their glycaemic control, as this could potentially make the results from this study invalid.

6.5 PARTICIPANT SELECTION, ENROLMENT AND WITHDRAWAL

6.5.1. IDENTIFYING PARTICIPANTS

Study staff, who are part of the diabetes care team in NHS Lothian, will identify potential participants who attend T1D clinic and from the Lothian diabetes research register and give them the opportunity to express an interest in taking part in the study. Electronic health records (Trakcare and SCI Diabetes) of potential participants will be screened for any exclusion criteria in order to save any unnecessary trips for those who would be excluded (Section 6.3).

If no exclusion criteria are identified, study staff will write to the potential participant inviting them to contact the research team by text or telephone, if they would like to know more about the study. This written correspondence will include a patient information leaflet (PIL) and copy of a consent form (CF) which they would sign at enrolment Visit 1 if they were taking part. Following patient and public involvement and engagement from individuals living with T1D we resolved to expand our initial PIL into an easier to digest PIL booklet which may be posted out to study participants or provided to them at Visit 1. We will also aim to create a webpage which study participants can access in the same format of information through a more accessible medium.

Potential participants will be encouraged to read the PIL booklet and CF, and discuss taking part in the study with family members, study staff and the doctors in charge of their routine clinical care before deciding whether or not to participate.

A designated mobile "dumb" phone has been sourced for the purposes of contacting and being contacted by research participants. Potential participants will be able to contact study staff by text to request a call back, or by telephone call, or can email Dr Tyndall on her university email address if they prefer. Additionally, our research group has it's own clinical email inbox. Participants will also be given the email contact for Professor Forbes on their patient information sheet in addition to the work and email contact details for an external member of staff (Dr Faye Baxter) who they can contact if they would prefer. Dr Baxter is a paediatrician who has previously undertaken hypoglycaemic clamp work with our group[13], and has kindly agreed to be a point of contact for potential participants as she will not be directly involved in the APH1D study or any clinical care of participants. Please see the patient information sheet, visit summary and recruitment flyer for full details.

From the date the PIL, CF and study staff contact information are provided, prospective participants will be given a minimum of five days and a maximum of 14 days to consider taking part in the study. If study staff are not contacted by the potential participant within this 14 day period, a member of study staff will follow-up with a telephone call. If the person is then agreeable to taking part in the study and considered suitable for enrolment, they will be invited to the CRF for screening/enrolment Visit 1 .

6.5.2. CONSENTING PARTICIPANTS

At Visit 1, the study protocol, PIL and CF will be discussed with the potential participant by study staff. Consent will be obtained, in person, in writing, by the study doctors at the start of study Visit 1 prior to proceeding with any further medical history, examination, ECG or blood testing (detailed in Table 1 and Figure 6)

6.5.3. WITHDRAWAL OF STUDY PARTICIPANTS

At each study visit, the investigators will confirm verbally with each participant that they continue to consent to take part in the study. Participants they will be reminded that they are free to withdraw from the study at any time. Out with study visits, participants will be directed to contact the investigators via text, call or email using the contact details in the PIL. They will be assured that they do not have to give any reason for withdrawing from the study, and that it will not impact their clinical care or follow-up in any way.

A participant can also be withdrawn by the chief investigator, and if this is done, the primary reason for withdrawal will be documented in the participant's case report form. To safeguard rights, the minimum personally identifiable information possible will be collected.

The participant may wish to withdraw from the study in one of two possible ways:

i. From all aspects of the study going forward. In this case, no further data will be collected. However, data collected up to that point may still be included in the final analysis (we will be proceeding on an "intention to treat" analysis as is best practice). For example, if a participant withdraws the day before Visit 3, blood samples stored from Visit 2 would be yet to be analysed, and so would be kept in storage for analysis alongside other samples.

ii. From every aspect of the study already completed. In this case, any blood samples still in storage will be properly disposed of by CRF staff at the instruction of the investigators, any electronic data collected would be deleted from both NHS and University Servers, and any paperwork which would not compromise Good Clinical Practice to be disposed of (e.g. adverse event reporting forms or correspondence to GP) would be disposed of via NHS confidential waste procedures.

6.6 PATIENT AND PUBLIC INVOLVEMENT

This section outlines the feedback received from individuals living with Type 1 Diabetes (T1D) not themselves eligible for inclusion in the APH1D study regarding the documentation provided to potential participants. The feedback has informed several key actions aimed at improving participant materials and study procedures.

Participants noted that the Patient Information Leaflet (PIL) contained a substantial amount of information, which could be overwhelming and challenging to digest. They suggested that the information be presented in several formats such as on a webpage, and in expanded booklet with easier to digest "chunks". To address this, present the same information in a professionally compiled booklet, organizing information into sections to facilitate easier navigation and reference for participants with expansion on a few points. Where possible, will also reflect the same information on a study webpage and ensure that study participants are given the information in an electronic as well as hard copy format to enhance accessibility.

Participants suggested the implementation of reminder texts to inform them of upcoming study visits, preparatory instructions, and any necessary insulin adjustments. We will introduce a system using the designated study phone to send these reminders, enhancing participant compliance and readiness.

PPI participants all raised concerns about the availability of replacements for sensors and cannulas, which could potentially disincentivise participation or cause stress on the day. Support for pump management post-clamp and provision of extra corrective supplies were suggested. We therefore will work with the local diabetes technology team to ensure that appropriate spares and replacements are provided in order to support continued participant involvement and address supply constraints which already exist within the NHS. We will provide a "goody bag" with hypo treatments and necessary spares after clamp visits to ensure participant safety and comfort.

Feedback indicated confusion regarding the number of cannulas used during the study. To improve understanding, document language will be revised to clearly and consistently describe the use of cannulas, ensuring participants have accurate expectations.

Participants requested clarity on the shopping vouchers provided and the definition of "local" concerning transportation services. We will specify the voucher details, including their value, expiry, and usability, and clearly define "local" as living within the Lothians area.

To address any potential fears about the clamp procedure, particularly regarding hypoglycaemia, information on safety measures and post-procedure checks will be included in the PIL. This aims to reassure participants of the study's safety.

In terms of advice following clamp experiment, PPI participants highlighted the need of flexibility for those with active jobs rather than a "one-size fits all" study schedule / instructions. In response, we will incorporate more adaptable elements into the study schedule, allowing investigators to tailor pre- and post-clamp advice to individual needs and ensure that this is documented on both the paper case report forms and in the electronic medical record.

These integrated measures aim to enhance participant experience, address logistical challenges, and improve overall study engagement.

7. STUDY ASSESSMENTS

If willing to partake in the study, participants will be invited to the CRF for an enrolment visit (Visit 1), where informed written consent will be obtained (Section 6.5.2), prior to a more detailed evaluation of eligibility and documentation of baseline characteristics. Table 1 details the assessments undertaken at each of the three visits to the CRF.

Table 1: APH1D Visit Summary of Assessments at each visit: There will be a phonecall between visits 1 and 2 to confirm eligibility and arrange the next study visit. We anticipate between 1 and 4 week gap between visits 1 and 2 and a 4 to 6 week gap between visits 2 and 3. However, the time between visits can be extended up to 12 weeks if required, without impact on the study integrity, provided study criteria are still satisfied.

Assessment	Visit 1: Enrolment	Visit 2: First Clamp	Visit 3: Second Clamp
Demographic data and contact details	☒		
Assessment of eligibility criteria	☒		
Observations: weight, height, BP, HR	☒	☒	☒
Written, informed consent	☒		
Full medical history and medication history	☒		
Physical examination ("A to E") and ECG	☒		
Recent CGM data for preceeding-14 days	☒	☒	☒
Randomisation by pharmacy		☒	
Blood samples for HbA1c, FBC, U&E, TFT, glucose and if required, C-peptide	☒		
Blood samples for reproductive hormones (LH, FSH, testosterone, progesterone and estradiol)	☒		
Urine sample for pregnancy test	☒	☒	☒
Urine sample for alb:creatinine	☒		
Arginine Stimulation Test (see Figure 6)	☒		
Blood samples for glucagon, cortisol (plus other steroid & reproductive hormones), adrenaline, noradrenaline, glucose and Aniracetam (see Figure 7)		☒	☒

7.1 VISIT 1: ENROLMENT AND ELIGIBILITY ASSESSMENT

Each potential participant will be evaluated for study eligibility to ensure that the clamp studies can proceed safely. This will be done through the elicitation of a full medical history, with a focus on Diabetes, plus a physical examination by study staff and local laboratory testing if needed to screen for exclusionary medical conditions. Any incidental findings (for example, a positive pregnancy test or new diagnosis of thyroid dysfunction), will be communicated to the potential participant, their GP and, if appropriate, their secondary care diabetes physician by telephone call and confidential mail.

The following data will be collected:

- Inclusion and exclusion criteria assessment (see Sections 6.2 and 6.3 of this document)
- Demographics (date of birth, sex, race and ethnicity)
- Contact information (retained at the site and not entered into study database)
- Comprehensive medical history
- Diabetes history, including scores for hypoglycaemia awareness, recent HbA1c, and previously treated complications (if any)
    - If retinopathy screening has not been carried out at an appropriate interval, it will be carried out at this visit or an appropriate date selected to suit the participant.
- Concomitant medications and any allergies
- Urine pregnancy test if appropriate
- Blood tests for full blood count (FBC), glycosylated haemoglobin (HbA1c), urea and electrolytes (U&Es), thyroid function gonadotrophins, estradiol, progesterone and testosterone.
- Arginine-stimulated C-peptide, glucagon, insulin and glucose using an arginine stimulation test (ArST) at visit 1
- CGM data: percentage time in glucose range 4-10 mmol/l (TIR), percentage time at or below glucose range 3.9 (TBR) mmol/l and glucose management index (GMI) for the two weeks preceding this visit; as well as “Snapshot” details of frequency and duration of hypoglycaemic episodes.
- Urine sample for albumin:creatinine ratio
- Physical examination

Observations and measurements Weight, height, BMI, vital signs including measurement of blood pressure and heart rate. ECG. Participants will be advised not to donate blood when recruited to the study, though less than 250 ml of blood will be taken by blood sampling during the whole of the APH1D study . The participant will be contacted by telephone once results of all tests are available to the study Drs to confirm eligibility and set a mutually suitable date for study visit 2. The participant will be allocated a study participant ID APH_00X e.g. APH_001, APH_002, etc.

Randomisation (1:1) will be carried out after recruitment at Visit 1 and between 1 and 7 days before the first hypoglycaemic clamp visit by the ERI NHS clinical pharmacists. The pharmacists will be unblinded and responsible for the allocation, dispensing and recording of Aniracetam and Placebo as detailed in Section 5.3.

7.1.1. Arginine Simulation Testing

Intravenous arginine stimulation testing (ArST) is increasingly used in clinical and translational settings to identify low-level residual β-cell function and to characterise α-cell (glucagon) responsiveness[26,27]. ArST reliably elicits rapid increases in insulin/C-peptide and glucagon, even when glucose-stimulated secretion is minimal[28]. Stimulated C-peptide has been shown to correlate with clinically meaningful

outcomes, including reduced frequency of hypoglycaemia and β-cell function in cystic fibrosis and islet transplant patients[29,30]. Only potential participants that demonstrate a random C-peptide level below the lower detection limit of the NHS assay (<50 pmol/l) and a paired with a plasma glucose level above 6 mmol/l will be recruited for visit 1.

By undertaking ArST at Visit 1, participants with residual insulin/C-peptide secretion, and by extension β-cell function (so called "microsecretors"), can be appropriately accounted for in this study. Colleagues in Copenhagen have undertaken similar experiments, and found a stimulated C-peptide and glucagon to peak at between 2 and 4 minutes following a 5g IV arginine infusion over 30 seconds[31]. Therefore we will measure insulin, C-peptide, glucose and glucagon at four time points relative to stimulation with arginine (Figure 6).

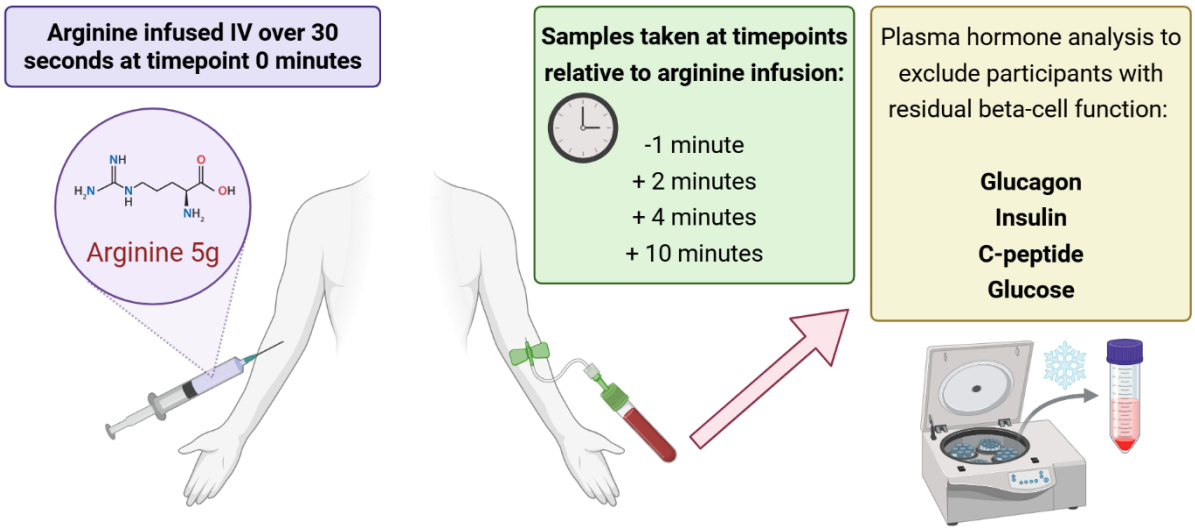


Figure 6: Arginine Stimulation Testing at Visit 1: A set of samples for glucagon, insulin, C-peptide and glucose are taken and one minute later, a solution of 5g Arginine is infused via an antecubital vein over 30 seconds. Blood for the same samples is then taken at 2, 4 and 10 minutes after the Arginine infusion is started. Samples are placed onto ice and centrifuged after completion of collection, and plasma stored at -80 degrees for later analysis.

7.2 RANDOMISATION

Following visit 1, an investigator will contact the participant by telephone to let them know whether they are eligible. If so, they will arrange a mutually convenient date for the second study visit to occur. Once this date is confirmed, the investigator will contact the clinical pharmacy team between one and seven days prior to visit 2 to let them know that the participant is eligible and ready for randomisation.

In addition to their unique study ID (e.g. APH_00X), each participant who undergoes randomisation will be designated a randomisation allocation number (e.g. RN_00X) by the clinical pharmacy team. They will do this by selecting the next randomisation code on the allocation list generated for them by our statistician.

Participants will be randomised to receive aniracetam or placebo in a 1:1 ratio. This will be stratified by sex to ensure as far as possible that equal numbers of males and females allocated to each group. We plan to recruit 50% males and 50% females into the study. The randomisation list determines the order of physiological testing and all participants receive both interventions. This paired within-participant

comparison allows the physiological responsiveness of the pathway to be assessed while minimising the number of participants required to undergo experimentation.

The working day before the clamp, the pharmacy team will dispense two tablets of either aniracetam or placebo into HDPE bottles. Each bottle will have an accompanying blinded prescription of "aniracetam/placebo" and pharmacy will deliver this prescription to the CRF for administration by investigators during the clamp. Appropriate accountability logs will be kept in accordance with GCP and GMP in the pharmacy site file.

At the end of the study, the Sponsor will notify the pharmacy team to lift the blinding.

7.3 STUDY VISITS 2 AND 3: HYPOGLYCAEMIC CLAMPS

Subject to successful enrolment into the APH1D study after visit 1, each participant will attend the CRF to undergo their first hypoglycaemic clamp at a mutually convenient date, in line with study timelines. This technique is safe and is considered, world-wide, as the gold standard approach in the assessment of hypoglycaemia[12]. Each clamp will take between three and four hours to complete. Insulin doses and CGM metrics of blood glucose data for the preceding 24 hours will be reviewed by the investigator before commencing, and an up-to-date list of current concomitant medications will be taken. Figure 7 gives an outline of our clamp protocol.

7.3.1. Participant preparation for clamp

In the 48 hours preceding the study participants will be asked to avoid moderate/high intensity exercise, defined as any exercise which may result in sweating or heavy breathing e.g. jogging, running, swimming and weight/resistance training[12,32,33]. They will also asked to be avoid alcohol and any medication that may affect blood glucose such as: salicylates (aspirin) and quinolone antibiotics, and to contact the research team to postpone the clamp study if taking an acute course of these medications is unavoidable. Participants will also be advised to avoid significant hypoglycaemia in the 48 hours before the study (defined as a glucose level below 3 mmol/l for more than 20 minutes) and that if this happens, the clamp will need to be postponed. Participants will be advised to contact us in advance of the clamp to avoid unnecessary travel to the ERI.

The day before the study, participants will be asked to do the following:

i. Avoid taking caffeine (tea, coffee, energy drinks, supplements etc) for 12 hours[34,35].
ii. Consume a moderate carbohydrate (40-60g) evening meal the night before
iii. Fast for at least 8 hours before presenting to the clinical research facility at 07:00 (participant can continue to consume water as required)
iv. Avoid hypoglycaemia- the study will be postponed if the blood glucose is <3mmol/l for >20 minutes
v. If on basal/bolus or open loop pump system to reduce their basal insulin the night before by 20% in order to avoid hypoglycaemia
vi. If on a closed loop system to place the pump on exercise settings the morning of testing

7.3.2. Postponing a hypoglycaemic clamp

The clamp study may be postponed by either the participant or the investigator for any one of the following reasons:

i. The participant has experienced significant hypoglycaemia in the preceding two weeks. This has two definitions:
   a. More than 4% time below range (a CGM glucose of 3.9 mmol/l or lower) **and** average time spent hypoglycaemic of longer than 20 minutes at a time on review of CGM metrics
   b. A significant hypoglycaemic event (defined glucose level below 3 mmol/l for 20 minutes or more) within the preceding 48 hours.
ii. The participant has been unable to adhere to the above guidance in Section 7.3.1 regarding exercise, caffeine, fasting and adjustment of the basal or background insulin doses in a way which would likely confound the study results
iii. The participant has recently given blood (it will be rescheduled until 12 weeks post donation if male and 16 weeks if female)
iv. The participant is taking a short course of medications which could interfere with the results of the study e.g. aspirin or other salicylates, quinolone antibiotics
v. Any unforeseen circumstance such as an acute illness or a life event which would make proceeding with the clamp impractical and/or could place the participant at risk of harm

If the participant finds that their clamp study must be postponed for any reason, they will be asked to contact Dr Tyndall on the mobile telephone which has been designated for use in the research study.

7.3.3. A hypoglycaemic clamp

An outline of our hypoglycaemic clamp protocol is given in Figure 7. When participants arrive in the ERICRF they will have their CGM metrics reviewed and their weight re-checked in order to calculate contemporary infusion rates accurately. They will also be asked to provide a urine sample for pregnancy testing if appropriate.

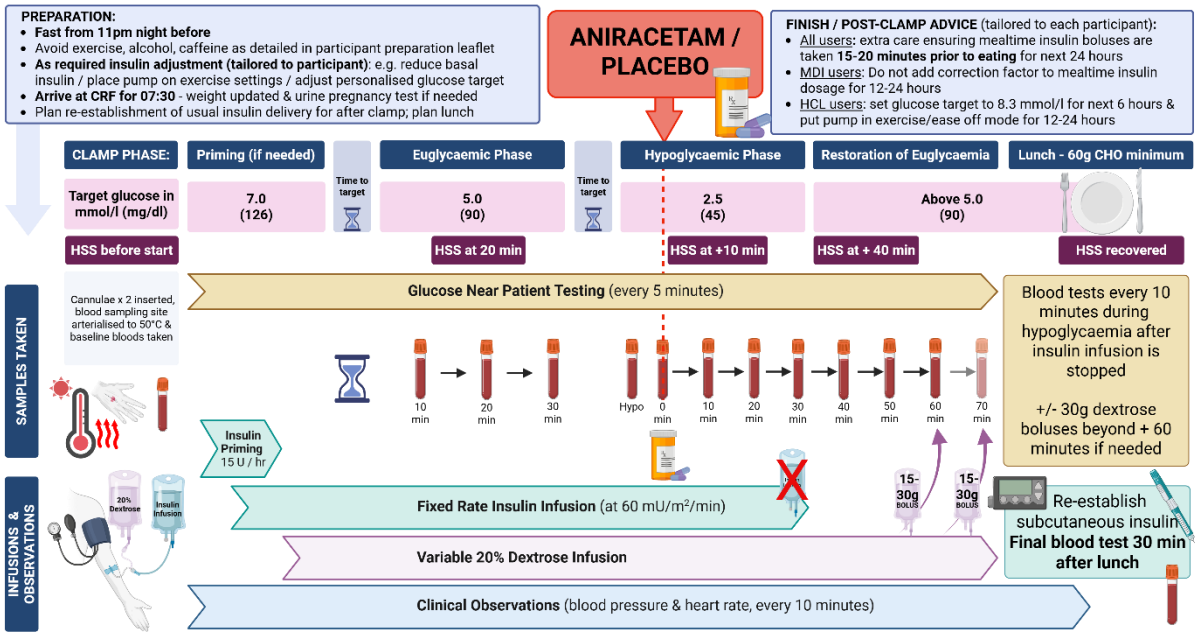


Figure 7: Hypoglycaemic Clamp Summary Diagram: During each clamp phase, arterialised blood sampling will be performed every 10 minutes for glucagon, cortisol, growth hormone, glucose, adrenaline, noradrenaline and Aniracetam until the clamp is over. See

Table 2 for a more detailed breakdown of blood samples being taken across the APH1D study.(CHO=carbohydrate; HSS=Hypoglycaemia symptom score; HCL=hybrid closed loop; MDI=multiple daily injection).

Table 1 details the blood tests which will be undertaken at each clamp study as well as the other blood tests performed at Visit 1 during enrolment. Two peripheral intravenous cannulas will be inserted. A cannula will be inserted into the dominant hand or antecubital fossa to be attached to both a fixed rate insulin infusion and variable rate 20% dextrose infusion (connected together by a dual lumen cannula).

A second cannula will be inserted into the non-dominant hand, which will then be placed in a heated blanket at 55°C in order to arterialise the venous blood at the point of sampling. A 3-way tap is employed for blood sampling from this site throughout the study. At each 5 minute collection, approximately 2.5ml of 'dead space' blood is withdrawn distally through a smart-site adaptor into a dedicated 5ml leuer-lock syringe. The tap is then adjusted to allow blood sampling through a monovette adaptor at the side port. After samples are obtained, the tap is readjusted, and the 'dead space' blood is re-injected into the participant. The smart site is then cleaned with an alcohol wipe and this is followed by a flush of 2.5ml 0.9% saline (given from a pre-prepared 5ml syringe). This syringe will be left attached to the cannula port until the next collection time point, where it will be used for dead space.

This method avoids participants having blood discarded unnecessarily, prevents haemodilution (and erroneous blood glucose readings), and maintains cannula patency. However, if there is any clinical concern over this dead space blood becoming clotted and unsuitable for re-injection, then this 2.5ml of blood will instead be discarded after blood samples are taken. This potential for discard is accounted for in the bottom of

Table 2.

An insulin infusion (Novorapid or Actrapid) will be made up in 0.9% Normal Saline at a concentration of 1 unit per 1 ml. If the participant's blood glucose is >10 mmol/l at the start of the study, a priming dose of insulin (usually 15 units/hr) may be given in order to bring their glucose down to 7 mmol/l. After this point, the infusion will be delivered according to body surface area, at a rate of 60mU/ m^2 /minute. Using the participants body surface area to calculate the infusion rate avoids overdosing insulin in overweight participants[12]. The body surface area will be calculated using the formula[13]:

$$\text{Body Surface Area } (m^2) = \sqrt{(\text{height } (cm) \times \text{weight } (kg))/3600}$$

Alongside the insulin infusion, a variable rate 20% dextrose infusion will be commenced. The rate of dextrose this will then be adjusted as required to obtain the desired blood glucose nadir.

There will be two blood glucose plateaus during the clamp study, lasting for 40-50 minutes: a euglycaemic plateau of 5mmol/l, followed by a hypoglycaemic plateau of 2.5mmol/l. Throughout the clamp study blood glucose will be monitored every 5 minutes using Point Of Care Testing (POCT) on a Biosen device (with a glucose Haemacue machine for backup) and the participant will remain blinded to their precise blood glucose level in order to avoid introducing bias during the experiment.

Blood will be drawn alongside glucose samples at ten-minute intervals during these two plateaus for measurement of: cortisol, adrenaline, noradrenaline, and glucagon levels. During and after the

hypoglyceamic plateau, additional samples will be taken to measure the levels of aniracetam and it's active metabolites, which will serve as a check that Aniracetam has indeed been administered after blinding is lifted. Aniracetam 1500mg or placebo will be given ten minutes into the hypoglycaemic plateau, which will be designated time = 0 minutes (Figure 7).

During the clamp procedure, blood pressure and heart rate will also be monitored every 10 minutes using automated observational devices. Symptoms of hypoglycaemia (if any) will be documented and scored at each phase of the clamp and after it has completed using the Edinburgh Hypoglycaemia Symptom Score (HSS) questionnaire. The participant may stop the clamp at any time if they wish, for example, if they develop symptoms of hypoglycaemia and find them intolerable, or if observations or symptoms reported during the clamp indicate to clinical investigators that they are unwell and that the experiment should be aborted.

7.3.4. After a clamp – restoration

IV insulin infusion will be stopped at +30 minutes after the aniracetam/placebo have been taken (Figure 7). The investigators will continue to take samples at ten minute intervals and if a euglycaemic glucose of 5.0 mmol/l has not been reached by +60 minutes following aniracetam/placebo, the participant will receive a 15-30g bolus of dextrose (75-150ml 20% dextrose) at the clinical investigator's discretion. This is consistent with guidance on the treatment of hypoglycaemia in the clinical setting

For example, if a participant's glucose level is still 2.9 mmol/L at 60 minutes, the clinical investigator would likely wish to give them a 30g bolus. In comparison, if their glucose level is 3.7 mmol/L at 60 minutes, a 15g bolus of dextrose would be sufficient to assist with normalising the glucose level.

Participants will and undergo continued blood sampling every 10 minutes until their glucose level is 5.0 mmol/l or higher (Figure 7). At the end of the experiment the participants will be assessed to ensure they have fully recovered from the clamp. They will be asked to re-apply and/or restart their insulin pump given lunch consisting of at least 60g of carbohydrate. Thirty minutes after their lunch they will have a final arterialised sample taken and their cannulas will then be removed.

They will receive post clamp advice tailored to their diabetes management. They may leave the CRF when blood glucose is stable and above 5 mmol/l (90 mg/dl) and those using insulin pumps will be advised to set a temporary high target or use the "ease off" function for the next 12 hours. Those who inject insulin using insulin pens will be advised to avoid using correction doses of insulin for the next 24 hours.

In total we will take not more than 631 ml of blood across all three visits and no more than 290 ml of blood per visit, as summarised in Table 2. We anticipate that for most participants, less than 350ml blood over the course of the whole study will be needed.

Table 2: Summary of blood sampling required during each study visit: Glucagon, insulin and C-peptide with paired plasma glucose are taken as part of an arginine stimulation test (ArST) at visit 1 to exclude participants who "microsecrete" insulin.

Blood test		Collection tube and size	(Approx) No. of times tested	Volume required (mL)	Total volume (mL)
Visit 1: Enrolment					
NHS Samples	HbA1c	EDTA 4.9 mL	1	4.9	4.9
	Full blood count				
	Creatinine, urea and electrolytes (U&Es)	Lithium-Heparin 4.9 mL	1	4.9	4.9
	Thyroid function testing (TFT)				
	Liver function testing (LFT)				
	Gonadotrophins (LH/FSH)				
Estradiol, Progesterone & Testosterone		Lithium-Heparin 4.9 mL	1	4.9	19.6
ArST C-peptide, insulin, glucose			4		
ArST Glucagon		EDTA 4.9 mL	4	4.9	19.6
Discarded blood (ArST)		2 mL	3	2	6
		Total blood taken at Visit 1:			**55 mL**
Visits 2 and 3: Hypoglycaemic Clamps					
Glucagon		EDTA	15-20	2.6	39 – 52
Cortisol and other hormones		Lithium-Heparin	15-20	4.9	74 – 98.0
Adrenaline / Noradrenaline					
Aniracetam + metabolites					
NPT-Glucose		Whole blood via slip-lock 1mL syringe	40-60	0.3	12 – 18
		Total blood for analysis per clamp			125 – 168
Potentially Discarded blood [¥]		*2 mL*	40 - 60	-	*80 - 120*
		Total blood taken (including discarded blood) **per clamp**:			**205 – 288 mL**
TOTALS					
Max amount of blood taken (excluding discarded blood) across whole study:					**305 – 391 mL**
Max amount of blood taken (including *potentially* discarded blood) across whole study:					**505 – 631 mL**

[¥] ***If there is any clinical concern over clotting (for example, if sampling the two EDTA and Lithium-Heparin tubes during the clamp is taking a longer time than expected), the above accounts for 2mL blood to be discarded rather than put back after blood samples are taken. This is with the exception of NPT glucose as this is a quick bed-side test.***

ArST=Arginine Stimulation Test; FSH=Follicle Stimulating Hormone; LH=Leutinising Hormone; NPT=Near patient Test

The samples will be immediately cooled (avoiding freezing) and processed within 20 minutes of collection in batches of two or three at a time. During the ArST and during each clamp, all samples will be centrifuged in a cool centrifuge at 2000g for 10 minutes after collection on site, before plasma supernatant is split into 250 µl aliquots and frozen in a dedicated monitored freezer at - 80°C . The blood samples will be stored in a secure and monitored environment and will be disposed of according to the Human Tissue Authority's code of practice after a period of 5 years.

7.3.5. Blinding of Aniracetam vs Placebo

After 4-6 weeks (though the period between study visits can be extended to 12 weeks if necessary, as long as study criteria are still satisfied) the participant will attend the ERICRF for their second hypoglycaemic clamp. They will be allocated to the group they were not in during the first clamp. (Aniracetam or placebo). The clinical pharmacy team members will be unblinded and in charge of distributing the tablets and will have no direct contact with the participants.

If the investigator believes that unblinding is essential to decide treatment for a serious adverse event following or during the clamp procedure, the pharmacist will be contacted and the clamp medication will be unblinded the same or the following working day. An out of hours code-break is not required as it would not affect the clinical management of any medical emergency or condition.

7.3.6. Discharge following clamp experiments and participant compensation

At the end of each hypoglycaemic clamp following recovery, participants will be given a lunch (consisting of at least 60g of carbohydrate) and "post-clamp advice" (see participant preparation checklists for full details). Each participant will receive a £100 shopping voucher per clamp (£200 in total) in addition to renumeration for transport costs (e.g. bus/train fare if needed or a pre-booked taxi) as per the University of Edinburgh policy.

7.3.7. Follow up assessments

There will be no long term follow up. However, each participant will be advised to adjust their insulin administration habits accordingly for 24 hours following each clamp study.The clinical investigator(s) will additionally review the CGM data of each participant at 48 hours following each clamp they have undertaken to monitor for any further episodes of hypoglycaemia. At 48 hours after the third visit (second clamp experiment) the participant will no longer be considered enrolled in the study. At this point, alerts in the participant's electronic patient record highlighting participation in the study will be deleted/inactivated.

7.3.8. Storage and analysis of samples

During the study period participant blood samples will be stored in the ERI CRF and either transferred to the NHS labs in the ERI for analysis or to our in-house lab in the QMRI for our own lab analysis. All blood samples will be stored in a secure and monitored environment and will be disposed of according to the Human Tissue Authority's code of practice after a period of 5 years. This is included in the consent form. In-house laboratory analysis will be carried out in Professor Forbes' research laboratory in the Queen's Medical Research Institute (QMRI), Little France.

7.3.9. Data collection

During the study, data and results will be collected and collated by study staff. The following data will be collected at these time points:

1) *Selection of potential participants prior to consent:*
   a. Review of electronic health care records, Trakcare and SCI Diabetes, and CGM data (using Libreview, Dexcom Clarity or Glooko platforms) for exclusion criteria
   b. This data will be reviewed by study staff who are part of the participant's healthcare team

2) *Eligibility Assessment (after obtaining written informed consent):*
   a. Inclusion and exclusion criteria assessment.
   b. Demographics (date of birth, sex, race and ethnicity)
   c. Contact information (retained at the site and not entered into study database)
   d. Medical history

e. Concomitant medications
f. Pregnancy test if applicable
g. HbA1c blood test
h. Bloods tests for: renal function thyroid function, reproductive hormones
i. CGM data: TIR, TBR and GMI and other CGM data for the two weeks preceding this visit (again using Libreview, Dexcom Clarity or Glooko platforms)
j. Urine sample for albumin:creatinine ratio
k. Physical examination to include Weight, height, BMI, vital signs including measurement of blood pressure and heart rate
l. Updated retinopathy screening if not completed within the last 12 months

3) First and second clamp studies

a. Weight
b. Blood pressure and heart rate
c. CGM data: TIR, TBR, snapshot and GMI for the CGM data for the two weeks preceding this visit (again using Libreview, Dexcom Clarity or Glooko platforms)
d. Blood glucose values from near testing
e. Infusion rates and quantities of insulin and dextrose during and between nadirs
f. Record of blood samples taken before and during clamp procedures

7.3.10. Source Data Documentation

Source documents for the study:

- Electronic health records: Trakcare and SCI Diabetes
- CGM data – Libreview, Dexcom Clarity, Glooko
- Participant case report forms
- Study questionnaires and consent forms
- Study master site files
- Case Report Forms
- Observation and infusion records during clamp experiments

7.4 Case Report Forms

All patient identifiers will be removed and allocated a unique study ID number e.g. APH_00X. Participant data which does not lend itself to be stored electronically (e.g. visit checklists, ECG readouts, written consent, or while filling out infusion protocols) will be collected on paper case report forms. The case report form will be identified using the participant's unique study identifier with no identifiable information such as name or CHI. The case report form will be stored in locked and secure rooms in the ERI. Electronic information containing patient identifiable information (e.g. name or CHI) will only ever be stored on an NHS computer with password-controlled access. Case report forms can be later uploaded into the participant's electronic patient record (EPR) under a research note if needed.

8. DATA MANAGEMENT

8.1 PERSONAL DATA

The following personal data will be collected as part of the research:

- Name
- Address

- Date of birth
- Contact telephone number(s)
- CHI number
- Email address

This is to ensure that research is carried out properly and that research investigators can appropriately access patient records. No identifiable information will be accessed, shared or stored outside NHS systems. This information will be accessed using an NHS password-protected computer with appropriate encryptions by study investigators.

8.2 DATA INFORMATION FLOW

Personal data collected by study staff will be held on secure password protected NHS computers in the Edinburgh Royal Infirmary and in a locked cupboard in the Edinburgh Royal Infirmary. Only study staff will have access to this data. Anonymised and pseudonymised data will additionally be held on secure password protected University of Edinburgh computers in the Unviersity of Edinburgh. Anonymised and pseudonymised reports on the study sent during the study period to the Helmsley Charitable Trust, whose headquarters are based in the United States.

8.3 DATA STORAGE

Hard copy documents which contain personal data of participants (e.g. CHI number on medication administration charts) will be stored by the research team in the Edinburgh Royal Infirmary under lock and key and on password protected NHS laptops. This data will only be accessible by study staff. At randomisation, participants will additionally be pseudonymised by a randomisation number (e.g. RN_00X) by clinical pharmacy team in order to maintain blinding. The code break for randomisation will be kept on a password protected NHS computer by Clinical Pharmacy team.

Electronic data, pseudonymised by unique study ID number (APH_00X) will be collated for later analysis using RedCap and Microsoft Excel. All data collected using Redcap will be anonymised/pseudonymised using the participant's unique study ID and accessed only using encrypted University of Edinburgh or NHS computer hardware. Anonymised and pseudonymised data will be stored securely in DataStore in University of Edinburgh for 10 years.

8.4 DATA RETENTION AND DISPOSAL

As stated above, electronic anonymised/pseudonymised data will be stored in the University of Edinburgh DataStore and will remain anonymised/pseudonymised after collection. Storage will be on the research group shared drive for 10 years. After this, if long term data preservation is required, data will be archived using DataVault. If no longer required, the data will be deleted in accordance data protection policy.

Hard copy study documentation will be kept under lock and key in OPD2 for 6 years from the protocol defined end of study point. After this point, hard copy documentation will be archived for a further 4 years before destruction, which will take place in accordance with NHS policy and data protection regulations.

8.5 EXTERNAL TRANSFER OF DATA

Identifiable data used by the study (including personal data) will not be transferred to any external individuals or organisations outside of the Sponsoring organisation(s).

Reports containing anonymised/pseudonymised data will be sent during and after the study period to the Helmsley Charitable Trust and may be shared with other academic institutions and groups including institutions outside of the UK/European Economic Area. This will only be done for purposes related to this study and other ethically approved research in line with GDPR and local data protection laws, and only then if participants have consented to this.

8.6 DATA CONTROLLER

A data controller is an organisation that determines the purposes for which, and the manner in which, any personal data are processed. The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site).

8.7 DATA BREACHES

Any data breaches will be reported to the University of Edinburgh (dpo@ed.ac.uk) and NHS Lothian (Lothian.DPO@nhslothian.scot.nhs.uk) Data Protection Officers who will onward report to the relevant authority according to the appropriate timelines if required.

8.8 DATA MONITORING COMMITTEE

The Data Monitoring Committee (DMC) will oversee data management practices, including the security and integrity of both hard copy and electronic data as well as safety reporting. The DMC ensures adherence to protocols for data retention, storage, and disposal, and may provide recommendations on data management processes to safeguard participant confidentiality and safety, in addition to study integrity.

9. STATISTICS AND DATA ANALYSIS

9.1 SAMPLE SIZE CALCULATION

A sample size of 18 participants would give 80% power to detect an effect size of 0.7 using a two-sided paired test at a 5% level of significance. Where the effect size is the absolute value of the difference in means in the active and placebo phase divided by the standard deviation of the difference.

There are no published studies in patients with T1D receiving Aniracetam that have studied the glucagon response to hypoglycaemia. However, in a study of 10 people with T1D versus 10 non-diabetic controls, the mean (SD) glucagon concentrations in response to hypoglycaemia were 32(9.5) vs 93(34.8) pg/mL. Assuming the glucagon concentrations in response to hypoglycaemia in that study in the participants with T1D are similar to what we will observe in our participants during the placebo phase and the control response will be closer to the response observed during our active phase although we do not expect it to be as high. With a SD of difference of 34.8 pg/mL and a mean of 32 pg/mL an effect size of 0.7 would equate to being able to detect a difference in means of 24.4 pg/mL i.e. a mean of 56.4 pg/mL.

9.2 PROPOSED ANALYSES

Primary analysis, will focus on the glucagon response when the AMPA/kainate pathway is modulated during insulin induced hypoglycaemia, subsequent recovery to euglycaemia and 30 minutes after a meal immediately following hypoglycaemia. By using a placebo tablet as a comparator, we will be able to perform a paired analysis. Secondary analysis will analyse glucose, growth hormone, cortisol, adrenaline and noradrenaline, as well as the Aniracetam levels measured in the blood.

It is important to note that statistical analysis is performed to describe physiological responses and generate hypotheses which may inform future research. No claims of therapeutic efficacy or safety for marketing authorisation can be made based on this data as aniracetam is (a) not available in the UK and (b) too short acting to have any clinical application people with T1D.

9.2.1. Summary measures to be reported:

- **Primary outcome measures:** Report mean hormone levels, along with standard deviations (SD)
- **Secondary measures:** Include the range and distribution of hormone levels across both normal glucose and hypoglycaemia phases.
- **Crossover data:** Differences in hormone levels between phases within individuals.

9.2.2. Method of analysis:

- **Comparative statistical analysis:** Use paired t-tests or repeated measures ANOVA to compare hormone levels during normal glucose and hypoglycaemic conditions, as study uses a crossover design.
- **Adjustments:** If necessary, adjustment for possible covariates for example age, sex, or baseline glucose levels, time to each glucose nadir and the dose of insulin and dextrose needed for each stage of clamp,using multivariable models.

9.2.3. Plans for handling missing, unused, and spurious data; non-compliers and withdrawals:

- **Missing data:** Use multiple imputation or last observation carried forward (LOCF) to handle missing hormone data, depending on the assumption about the missing data mechanism.
- **Unused or spurious data:** Identify and remove outliers based on predefined criteria (e.g., values greater than 3 SD from the mean)
- **Non-compliers:** Analyse non-compliers separately to assess any potential biases introduced by deviations from the protocol.
- **Withdrawals**: Use intention-to-treat (ITT) principles to include data from all participants who were randomized, even if they withdraw, using their last available data for analysis.

9.2.4. Plans for pre-defined subgroup analyses:

- **Subgroups**: Analyse hormone responses by predefined subgroups, such as:
    - Sex
    - Age group
    - Baseline glucose or insulin sensitivity levels
- **Statistical methods:** Use interaction terms in regression models or stratified analysis to explore differential effects across these subgroups.

9.2.5. Statement regarding use of intention to treat (ITT) analysis:

ITT principle: ITT analysis will be conducted, where all participants randomized will be included in the final analysis, regardless of withdrawal or non-compliance, ensuring unbiased results reflective of the crossover design.

10. ADVERSE EVENTS

An Adverse Event (AE) relates to any untoward medical occurrence which is NOT necessarily caused by the study intervention. An Adverse Reaction (AR) relates to a type of AE where an untoward or unintended response has occurred due to the study intervention. AEs are collected from time of consent up to two days after the final study visit (Visit 3).

A serious adverse event (SAE) is an AE(R) which:

- results in death of the participant;
- is life threatening*;
- requires in-patient hospitalisation^ or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect;
- results in any other significant medical event not meeting the criteria above.

**Life-threatening in the definition of an SAE(R) refers to an event where the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.*

*^Any hospitalisation that was planned **prior to enrolment** will not meet SAE criteria. Any hospitalisation that is planned post enrolment will meet the SAE criteria*

10.1ADVERSE EVENT REPORTING

When an adverse event occurs, the Investigator, or other suitably qualified person in the research team who is delegated to record and report AE(R)s, will review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. All AE(R)s will be recorded in an individual AE(R) log kept in each per participant folder.

Every AE(R) must be assessed for seriousness, causality, and severity. Any possibly related events must be assessed for expectedness by the Principal Investigator or another suitably qualified physician in the research team who has been delegated this role. For this randomised double-blinded study, all AE(R)s will be assessed as though the participant is taking active medication.

Information collected in the per-participant AE(R) log includes the type of event, onset (start) date, Investigator assessment of severity and causality, expectedness (if relevant), date of assessment, whether resolved or ongoing, date of resolution and who recorded by.

Participants will be asked about the occurrence of AE(R)s at every visit during the study. Open-ended and non-leading verbal questioning of the participant will be used to enquire about AE(R) occurrence. Participants will also be asked if they have been admitted to hospital, had any accidents, used any new medicines or changed concomitant medication regimens. If there is any doubt as to whether a clinical observation is an AE(R), the event will be recorded.

This study involves only people with T1D on insulin for whom hypoglycaemia is often a normal part of day-to-day life. Therefore, hypoglycaemia will not be recorded an AE(R) unless it is considered clinically significant i.e. it is a hypoglycaemic episode characterised by altered mental and/or physical

status requiring assistance from another person for treatment or requiring emergency medical intervention or hospital admission.

The participant will have the investigator's contact details to report any potential adverse effects at any time 9-5pm, from the point of informed consent up to 2 days after the final clamp procedure. Out of hours, if urgent, the participant will be advised to contact the GP out of hours service and to inform them of their involvement in the study. The GP will have received a letter from our team detailing the participant's involvement in the study and alerts will be placed on the participant's EPR for the duration of their participation in the study, so that if they attend hospital, the study team will be notified.

10.2SERIOUS ADVERSE EVENT REPORTING

For Serious AEs (SAE), an assessment of expectedness against the SmPC will be undertaken by checking the event against those listed in Section 4.8 of the SmPC "Reference Safety Information" (RSI).

Once the Investigator becomes aware that an SAE has occurred in a study participant, the information will be reported to the ACCORD Research Governance **within 24 hours**. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before notifying ACCORD. The SAE report can be updated when the additional information is received. Reports will be complete as far as possible and will be signed and dated by the Investigator.

The SAE form will be transmitted via email to safety@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

Once an SAE report is received by ACCORD it will be entered onto the ACCORD PhV database by the Research Governance Coordinator or designee. All safety reports transmitted to ACCORD (including any follow-up information and correspondence) will be kept by the Investigator in the Study Master File.

10.3SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION REPORTING

Any AR classed as serious, which is also suspected to have been caused by the study intervention but is unexpected as the event is not consistent with SmPC 4.8 RSI, will be classed as a Suspected Unexpected Serious Adverse Reaction (SUSAR). The same bespoke SAE form will be completed and SAE reported to ACCORD as detailed above in Section 10.2. In addition, the SUSAR form will be reported to the REC by ACCORD. This is different to CTIMP studies, where SUSAR reporting is to the regulator (typically the MHRA). Occurrence of a SUSAR will additionally be reported to the chair of the DMC to allow them to convene the committee if appropriate.

10.4UNBLINDING

Unblinding will be triggered if a serious adverse event is **related** *and* **unexpected**. An SAE report will be sent to the ACCORD Pharmacovigilance Team by investigators within 24 hours of the event occurring. Members of the Data Monitoring Committee will also be copied in to this email as they may wish to convene the committee. The ACCORD Pharmacovigilance Team will then contact the NHS clinical pharmacy team (loth.pharmacyclinicaltrials@nhs.scot) to request unblinding takes place and

report the SAE and unblinding to the REC within two weeks. An out of hours code-break is not required for this study as it would not affect the clinical management of any medical emergency or condition.

10.5URGENT SAFETY MEASURES (USM)

An Urgent Safety Measure (USM) is "*An immediate deviation from, or change to the protocol to eliminate immediate hazard to participants*". PIs must notify CIs and ACCORD immediately of any USM they have implemented. The CI must then notify the REC and ACCORD, in writing, within 3 days of the incident

10.6SAFETY EVENT FOLLOW UP

The PI is responsible for the follow up of participants until resolution of event or death of participant. An SAE report will not be considered complete until the outcome meets one of four critiera:

- The participant is completely recovered (including date of recovery)
- The participant is recovered with sequelae (including date of recovery)
- The participant has died (including date of death).
- If the diagnosis or event description has changed as a result of follow-up, then expectedness should be reassessed by the site PI.

11. OVERSIGHT ARRANGEMENTS

11.1 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit study related monitoring and audits on behalf of the Sponsor, REC review, and regulatory inspection(s). In the event of audit or monitoring, the Investigator agrees to allow the representatives of the Sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

11.2 STUDY MONITORING AND AUDIT

The ACCORD Sponsor Representative has assessed this research study as requiring "light" monitoring following a study specific risk assessment. The Monitor will perform monitoring activities in accordance with the study monitoring plan and completed a Site Initiation Visit on 13th April 2026, with the study team and research staff present. A first in-person monitoring visit is planned for after the first two participants have completed all three study visits. Additional monitoring visits may involve on-site visits and remote monitoring activities as necessary. ACCORD QA personnel, or designees, will perform study audits in accordance with the study audit plan. This will involve investigator site audits, study management audits and facility (including 3rd parties) audits as necessary.

A close out visit will be conducted remotely after the last participant's last visit. As later laid out in Section 13.5 of this document, the study team will contact the Sponsor and local R&D when the study ends and the CI will complete a Declaration of End of Study form. This will be sent to the REC and

Sponsors within 90 days of the study ending (or within 15 days of the study ending if it is terminated early). A copy of this will be included in reporting to the funder at the relevant time.

11.3 STUDY STEERING COMMITTEE

A Study Steering Committee will be established to oversee the conduct and progress of the trial. The terms of reference of this committee, the draft template for reporting and the names and contact details will be detailed in the relevant Committee Charters.

11.4 DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established to oversee the safety of participants in the study. The terms of reference of the Data Monitoring Committee and the names and contact details will be detailed in DMC & TSC Charters. These charters will be signed by the appropriate individuals prior to the study commencing.

12. GOOD CLINICAL PRACTICE

12.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (International Conference on Harmonisation GCP). Before the study can commence, all necessary approvals will be obtained, and any conditions of approvals will be met.

12.2 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff. Delegated tasks must be documented on a Delegation Log and signed by all those named on the list prior to undertaking applicable study-related procedures.

12.2.1. Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any study specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information – appropriate Participant Information Leaflets (PIL) and informed Consent Forms (CF) will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the Sponsor(s) for this study and other future ethically approved research. The consent form will include permissions for their personal information to be retained on NHS servers and passed to the University of Edinburgh for the purposes of administration of the study, which has been confirmed as requiring "Light" monitoring by the Sponsor. The participant will be informed of their unique study patient identification number (PIN), which will take the format APH_00X.

The participant will be informed and agree to their General Practitioner to be contacted and informed of their participation in the study. They will also agree for their next of kin (noted in their electronic patient record) to be contacted by investigators in the event that they cannot be reached and all reasonable efforts have been made to contact them. This is important if there, for example, an unexpected abnormal result returns (e.g. a new diagnosis of hypothyroidism) which requires immediate action.

Regarding their blood samples and data collected from their participation in this study, participants will consent to their pseudonymised/anonymised data being sent to other academic institutions within and outside of the UK / European Economic Area for purposes related to this study and other ethically approved academic research. This allows discussion of unpublished data and results with our international collaborators and reporting to our funder which is based in the USA.

The Investigator or delegated member of the study team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The original will be signed in the Investigator Site File (ISF). The participant will receive a copy of the signed consent form and a copy will be filed in the participant's medical notes.

12.2.2. Study site staff

The Investigator must be familiar with the protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the protocol and their study related duties.

12.2.3. Data recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site.

12.2.4. Investigator documentation

The Principal Investigator will ensure that the required documentation is available in local Investigator Site files (ISFs).

12.2.5. GCP Training

For non-CTIMP (i.e. non-drug) studies all researchers are encouraged to undertake GCP training in order to understand the principles of GCP. This is not a mandatory requirement unless deemed so by the Sponsor. GCP training status for all investigators should be indicated in their respective CVs.

12.2.6. Data Protection Training

All University of Edinburgh employed researchers and study staff will complete the Data Protection Training through Learn.

NHS Lothian employed researchers and study staff will comply with NHS Lothian mandatory Information Governance Data Protection training through LearnPro.

Non-NHS Lothian staff that have access to NHS Lothian systems will familiarise themselves and abide by all NHS Lothian IT policies, as well as employer policies

12.2.7. Information Security Training

All University of Edinburgh employed researchers, students and study staff will complete the Information Security Essentials modules through Learn and will have read the minimum and required reading setting out ground rules to be complied with.

NHS Lothian employed researchers and study staff will comply with NHS Lothian mandatory Information Governance IT Security training through LearnPro.

Non-NHS Lothian staff that have access to NHS Lothian systems will familiarise themselves and abide by all NHS Lothian IT policies, as well as employer policies

12.2.8. Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished information, which is confidential or identifiable, and has been disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

12.2.9. Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the appropriate data protection legislation (including the General Data Protection Regulation and Data Protection Act) with regard to the collection, storage, processing and disclosure of personal information. Computers used to collate the data will have limited access measures via user names and passwords. Published results will not contain any personal data that could allow identification of individual participants.

13. STUDY CONDUCT RESPONSIBILITIES

13.1 PROTOCOL MODIFICATIONS (PREVIOUSLY KNOWN AS AMENDMENTS)

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Proposed protocol modifications (previously known as amendments) will be submitted to the Sponsor for classification, review and authorisation.

Modifications to the protocol must be submitted in writing to the appropriate REC and local R&D for approval prior to implementation and prior to participants being enrolled into the amended protocol.

13.2 MANAGEMENT OF PROTOCOL NON COMPLIANCE

13.2.1. Protocol Waivers

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the Sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC and local R&D for review and approval if appropriate.

13.2.2. Management of Deviations and Violations

Deviation - Any change, divergence, or departure from the study design, procedures defined in the protocol or GCP that does not significantly affect a subjects rights, safety, or well-being, or study outcomes.

Violation - A deviation that may potentially significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being.

Deviations and violations are non-compliance events discovered after the event has occurred. Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the Sponsors every 3 months (quarterly). Each protocol violation will be reported to the Sponsor within 3 days of becoming aware of the violation. Deviation logs will be maintained for each site in multi-centre studies.

Deviation logs/violation forms will be transmitted via email to QA@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Forms may also be submitted by hand to the office. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

13.3 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the study; or

(b) the scientific value of the study.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the Sponsor(s) (**qa@accord.scot**) must be notified within 24 hours. It is the responsibility of the Sponsor(s) to assess the impact of the breach on the scientific value of the study, to determine whether the incident constitutes a serious breach and report to research ethics committees as necessary.

13.4 STUDY RECORD RETENTION

See Section 7: "Data Management" of this protocol for full details on study record retention.

13.5 END OF STUDY

The end of study is defined as 48 hours after the last participant's last visit. This is because investigators will be checking for any subsequent AE(R)s which may have occurred following the participant's

second hypoglycaemic clamp experiment. The Investigators and/or the Sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, and R&D Office(s) and Sponsor(s) within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the Sponsor(s) via email to researchgovernance@ed.ac.uk.

A summary report of the study will be provided to the REC and Sponsors within 1 year of the end of the study.

13.6 CONTINUATION OF TREATMENT FOLLOWING THE END OF STUDY

It is not possible to continue the treatment following the end of the study – the study involves mechanistic assessment of glucagan release only.

13.7 INSURANCE AND INDEMNITY

The Sponsor(s) are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the Sponsor(s)' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The Sponsor(s) require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's National Health Service will have the benefit of NHS Indemnity.
- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.

13.8 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team.

14. REFERENCES

1 Bell, K. J. & Lain, S. J. The Changing Epidemiology of Type 1 Diabetes: A Global Perspective. *Diabetes Obes Metab* **27 Suppl 6**, 3-14 (2025). https://doi.org/10.1111/dom.16501

2 Parekh, W. A., Ashley, D., Chubb, B., Gillies, H. & Evans, M. Approach to assessing the economic impact of insulin-related hypoglycaemia using the novel Local Impact of Hypoglycaemia Tool. *Diabet Med* **32**, 1156-1166 (2015). https://doi.org/10.1111/dme.12771

3 Berthon, W. *et al.* Ongoing burden and recent trends in severe hospitalised hypoglycaemia events in people with type 1 and type 2 diabetes in Scotland: A nationwide cohort study 2016-2022. *Diabetes Res Clin Pract* **210**, 111642 (2024). https://doi.org/10.1016/j.diabres.2024.111642

4 Husain, K. H. *et al.* Dementia in Diabetes: The Role of Hypoglycemia. *Int J Mol Sci* **24** (2023). https://doi.org/10.3390/ijms24129846

5 Cryer, P. E. Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. *Diabetologia* **45**, 937-948 (2002). https://doi.org/10.1007/s00125-002-0822-9

6 Gerich, J. E., Langlois, M., Noacco, C., Karam, J. H. & Forsham, P. H. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* **182**, 171-173 (1973).

7 Cryer, P. E. Minireview: Glucagon in the pathogenesis of hypoglycemia and hyperglycemia in diabetes. *Endocrinology* **153**, 1039-1048 (2012). https://doi.org/10.1210/en.2011-1499

8 Panzer, J. K., Tamayo, A. & Caicedo, A. Restoring glutamate receptor signaling in pancreatic alpha cells rescues glucagon responses in type 1 diabetes. *Cell Rep* **41**, 111792 (2022). https://doi.org/10.1016/j.celrep.2022.111792

9 Brissova, M. *et al.* alpha Cell Function and Gene Expression Are Compromised in Type 1 Diabetes. *Cell Rep* **22**, 2667-2676 (2018). https://doi.org/10.1016/j.celrep.2018.02.032

10 Cabrera, O. *et al.* Glutamate is a positive autocrine signal for glucagon release. *Cell Metab* **7**, 545-554 (2008). https://doi.org/10.1016/j.cmet.2008.03.004

11 Camunas-Soler, J. *et al.* Patch-Seq Links Single-Cell Transcriptomes to Human Islet Dysfunction in Diabetes. *Cell Metab* **31**, 1017-1031 e1014 (2020). https://doi.org/10.1016/j.cmet.2020.04.005

12 Fabricius, T. W. *et al.* Hyperinsulinaemic-hypoglycaemic glucose clamps in human research: a systematic review of the literature. *Diabetologia* **64**, 727-736 (2021). https://doi.org/10.1007/s00125-020-05361-8

13 Baxter, F., Baillie, N. & Forbes, S. Study protocol: a randomised controlled proof-of-concept real-world study - does maximising time in range using hybrid closed loop insulin delivery and a low carbohydrate diet restore the glucagon response to hypoglycaemia in adults with type 1 diabetes? *BMJ Open* **12**, e054958 (2022). https://doi.org/10.1136/bmjopen-2021-054958

14 I Ito, S. T., A Kohda, H Sugiyama. Allosteric potentiation of quisqualate receptors by a nootropic drug aniracetam. *The Journal of Physiology* (1990).

15 Lee CR, B. P. Aniracetam. An Overview of its Pharmacodynamic and Pharmacokinetic Properties, and a Review of its Therapeutic Potential in Senile Cognitive Disorders. *Drugs Aging* **4**, 257–273 (1994).

16 Himori, N., Suzuki, T. & Ueno, K. Aniracetam, a pyrrolidinone-type cognition enhancer, attenuates the hydroxyl free radical formation in the brain of mice with brain ischaemia. *J Pharm Pharmacol* **47**, 253-258 (1995). https://doi.org/10.1111/j.2042-7158.1995.tb05790.x

17 Nair, A. B. & Jacob, S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* **7**, 27-31 (2016). https://doi.org/10.4103/0976-0105.177703

18 Administration, T. F. a. D. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. (Silver Spring, MD, 2005).

19 Mayersohn M, R. G., Wendt G. Disposition Pharmacokinetics and Metabolism of Aniracetam in animals. *Drug Investigation* **5**, 73-95 (1993).

20 Cai S, W. L. Determination of aniracetam's main metabolite, N-anisoyl-GABA, in human plasma by LC-MS/MS and its application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* **897**, 50-54 (2012).

21 Lavipharm. *https://lavipharm.com/*.

22 Tian Y, Z. J., Feng SD, Zhang ZJ, Chen Y. Pharmacokinetics and bioequivalence study of aniracetam after single-dose administration in healthy Chinese male volunteers. *Arzneimittelforschung.* **58**, 497-500 (2008).

23 LeRoith, D. *et al.* Comparison of the counterregulatory hormone response to semisynthetic human insulin and purified porcine insulin in normal subjects and patients with type I diabetes mellitus. *Clin Ther* **13**, 613-626 (1991).

24 Davis, S. N., Shavers, C. & Costa, F. Gender-Related Differences in Counterregulatory Responses to Antecedent Hypoglycemia in Normal Humans1. *The Journal of Clinical Endocrinology & Metabolism* **85**, 2148-2157 (2000). https://doi.org/10.1210/jcem.85.6.6641

25 Galassetti, P. *et al.* Sexual dimorphism in counterregulatory responses to hypoglycemia after antecedent exercise. *J Clin Endocrinol Metab* **86**, 3516-3524 (2001). https://doi.org/10.1210/jcem.86.8.7720

26 Christensen, M. *et al.* Glucagon-like peptide-2, but not glucose-dependent insulinotropic polypeptide, stimulates glucagon release in patients with type 1 diabetes. *Regul Pept* **163**, 96-101 (2010). https://doi.org/10.1016/j.regpep.2010.05.004

27 Robertson, R. P. *et al.* Arginine is preferred to glucagon for stimulation testing of β-cell function. *Am J Physiol Endocrinol Metab* **307**, E720-727 (2014). https://doi.org/10.1152/ajpendo.00149.2014

28 Uitbeijerse, B. S., Nijhoff, M. F. & de Koning, E. J. P. Comparison of an oral mixed meal plus arginine and intravenous glucose, GLP-1 plus arginine to unmask residual islet function in longstanding type 1 diabetes. *American Journal of Physiology-Endocrinology and Metabolism* **326**, E673-E680 (2024). https://doi.org/10.1152/ajpendo.00030.2024

29 Sheikh, S. *et al.* Early-phase insulin secretion during mixed-meal tolerance testing predicts β-cell function and secretory capacity in cystic fibrosis. *Front Endocrinol (Lausanne)* **15**, 1340346 (2024). https://doi.org/10.3389/fendo.2024.1340346

30 Robertson, R. P. *et al.* Assessment of β-cell mass and α- and β-cell survival and function by arginine stimulation in human autologous islet recipients. *Diabetes* **64**, 565-572 (2015). https://doi.org/10.2337/db14-0690

31 Lund, N. E. S. a. A. B. (ed Victoria Tyndall) (2025).

32 Bird, S. R. & Hawley, J. A. Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport Exerc Med* **2**, e000143 (2016). https://doi.org/10.1136/bmjsem-2016-000143

33 Dubé, J. J., Allison, K. F., Rousson, V., Goodpaster, B. H. & Amati, F. Exercise dose and insulin sensitivity: relevance for diabetes prevention. *Med Sci Sports Exerc* **44**, 793-799 (2012). https://doi.org/10.1249/MSS.0b013e31823f679f

34 Moon, S. M., Joo, M. J., Lee, Y. S. & Kim, M. G. Effects of Coffee Consumption on Insulin Resistance and Sensitivity: A Meta-Analysis. *Nutrients* **13** (2021). https://doi.org/10.3390/nu13113976

35 Shi, X., Xue, W., Liang, S., Zhao, J. & Zhang, X. Acute caffeine ingestion reduces insulin sensitivity in healthy subjects: a systematic review and meta-analysis. *Nutrition Journal* **15**, 103 (2016). https://doi.org/10.1186/s12937-016-0220-7