Clinical Study Protocol

The HYPO-AVOID STUDY

Low-Dose Glucagon and Advanced Hybrid Closed-Loop System for Prevention of Exercise-Induced Hypoglycemia in People with Type 1 Diabetes

Lav-dosis glukagon og avanceret hybrid closed-loop system til forebyggelse af motionsinduceret hypoglykæmi hos personer med type 1 diabetes

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Steno Diabetes Center Copenhagen,
Clinical Research, Diabetes Technology Research

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Sponsor: Kirsten Nørgaard, MD, DMSc

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The sponsor, Kirsten Nørgaard and the primary investigator, Sissel Lundemose, guarantees that the study will be conducted in accordance with this protocol and current legislation and regulatory (GCP) requirements.

Date: 15.10.2021

Kirsten Nørgaard, MD, DMSc, Sponsor

Sissel Lundemose, MD, Primary investigator

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0. Abbreviations

T1D	Type 1 Diabetes
AHCL	Advanced Hybrid Closed-Loop
S.C.	Subcutaneous
CPET	Cardiopulmonary Exercise Test
BGM	Blood Glucose Monitoring
PG	Plasma Glucose
SG	Sensor Glucose
MICE	Moderate Intensity Continuous Exercise
CGM	Continuous Glucose Monitoring
eCRF	Electronic Case Report Form

1.Background and rationale

Type 1 diabetes (T1D) is a chronic disease caused by autoimmune destruction of the pancreatic insulin-producing cells which necessitates a lifelong reliance on exogenous insulin to achieve glycemic control (1). Near-normalization of blood glucose levels through intensive insulin therapy reduces the risk of diabetes related complications (2,3), but balancing exogenous insulin administration with the varying daily activities and everchanging physiological requirements remains a significant challenge.

In people with T1D, regular exercise is associated with improved glycemic control, reduced insulin requirements, bettered weight management and a lower prevalence of hypertension, dyslipidemia and microvascular complications (4).

However, managing blood glucose levels during exercise is difficult due to absence of the regulatory mechanisms that ensure stabile glycemic conditions in people without diabetes. In people without diabetes, physical activity leads to reduced endogenous insulin secretion and increased glucagon and catecholamine secretion which stimulates hepatic glucose release. This hormonal interplay helps facilitate equilibrium in the rate of intramuscular glucose supply to its increasing demand. In people with T1D, circulating insulin levels cannot be physiologically downregulated and the counterregulatory glucagon secretion is blunted (5). Consequently, engaging in aerobic exercise often induces low blood glucose levels (hypoglycemia).

Despite the established health benefits conveyed by physical activity for people with T1D (6–10), participation rates remain low, with fear of hypoglycemia, lack of freedom to engage in unplanned activities, and uncertainty in making appropriate adjustments to insulin and nutritional therapy reported as the leading barriers to regular exercise engagement (11,12).

New commercially available artificial pancreas systems, also known as ‘’advanced hybrid-closed-loop’’ (AHCL) systems, have shown great result concerning improvement of the glycemic target. The AHCL system continuously anticipates insulin needs, adjusts insulin delivery based on circulating glucose levels and help to protect the user from low glucose levels. However, the person with type 1 diabetes utilizing AHCL systems still needs to plan for exercise, i.e. announcing exercise to the algorithm in advance, and may still require conscious action to consume supplementary carbohydrates around exercise to prevent hypoglycemia.

An ongoing project at Steno Diabetes Center Copenhagen with the title Steno780G study is testing the integration of the MiniMed 780G system in people with T1D around exercise. The study is exploring how best to optimize glycemia around physical exercise through the application of different carbohydrates strategies in people with T1D treated with AHCL therapy. However, increased carbohydrate intake prior to, or during, exercise can negate some of the positive effect of exercise on weight management. Hence, alternative preventative or indeed treatment strategies that increase glucose without the associated caloric expense, may prove valuable for many people with T1D.

In recent years, a number of studies have successfully demonstrated that subcutaneous (s.c.) low-dose glucagon can be utilized to effectively treat insulin-induced and exercise-induced mild hypoglycemia in people with T1D (13–15). Likewise, s.c. infusion of low-dose glucagon in dual-hormone AHCL systems has shown promising results in people with T1D (9).

This will be the first feasibility/pilot study to evaluate the use of glucagon provision for hypoglycemia prevention during exercise in people with type 1 diabetes using AHCL therapy; the **‘’HYPO-AVOID’’** study. The results of this research may contribute useful data that helps identify glycaemic management strategies that encourage patient safety, and greater exercise participation in people with T1D.

2. Hypothesis

We hypothesize that low-dose glucagon administered s.c. before aerobic exercise is superior to no glucagon regarding glucose control around exercise in people with T1D using AHCL therapy.

3. Study objective

To compare the effect of low-dose glucagon (single 150 µg dose) administered immediately before aerobic exercise versus a control trial without glucagon on glucose responses during and after exercise in individuals with AHCL-treated T1D.

4. Study design

The HYPO-AVOID study consists of a screening visit (visit A) and two additional study visits (visit B and visit C) completed in a sequential order. During study visit B (detailed in section 9), participants will receive s.c. administration of 150 ug glucagon prior to a 45-min moderate intensity continuous exercise session and subsequently continue into a 1-hour post-exercise observation period. During study visit C the exact same procedures will be followed with the omission of glucagon.

5. Subject selection

The HYPO-AVOID study will include individuals with AHCL-treated (Minimed Medtronic 780G) T1D, and the participants will be recruited from the clinic at Steno Diabetes Center Copenhagen.

A written participant information will be handed out along with the brochure: "Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt".

For people interested in participating in the HYPO-AVOID study, a subsequent consultation will be scheduled and spoken information will be given by the investigator in a quiet environment. The investigator will, in written and spoken words, ensure that the participant is adequately informed about the study rationale and design. The participant will have the opportunity to ask questions and bring a companion to the interview. Before signing the consent form, the participant is given up to seven days to reconsider. Should the participant need further time, a follow-up meeting will be scheduled. The participants are informed that they may, at any time, withdraw their informed consent to participate in the study without this having consequence for their future treatment. The informed consent procedure may be scheduled at the same day as the screening visit (visit A); however, no study-related examinations will be conducted before the informed consent form has been signed.

In case a participant wants additional information about the study before or after giving consent, the sponsor and primary investigator can be contacted. Information details are given in the study information letter.

If the study is prematurely terminated, the investigator will promptly inform the study subjects and assure appropriate follow-up. The investigator will further inform the Regional Scientific Ethics Committee and the Danish Medicines Agency.

5.1 Inclusion criteria

- Age ≥ 18 years
- Type 1 diabetes ≥ 2 years
- Using the AHCL system MiniMed 780G ≥ 4 weeks
- Novorapid use ≥1 week

5.2 Exclusion criteria

- Allergies to lactose or glucagon
- Known or suspected allergies to glucagon or related products
- History of hypersensitivity or allergic reaction to glucagon or lactose
- Patients with diagnosed pheochromocytoma, insulinoma or gastroparesis
- Concomitant medical or psychological conditions identified through review of medical history, physical examination and clinical laboratory analysis that, according to the investigator's assessment, makes the individual unsuitable for study participation
- Lack of compliance with key study procedures at the discretion of the investigator
- Females of childbearing potential who are pregnant, breast-feeding or intend to become pregnant or are not using adequate contraceptive methods (methods are considered adequate for study enrolment for females: an intrauterine device, hormonal contraception (birth control pills, implant, patch, vaginal ring or injection), a single partner who is sterile or infertile, or sexual abstinence. Contraception is required throughout the study duration. Sterilized or postmenopausal women (>12 months since last period) are not required to use contraception)
- Inability to understand the individual information and to give informed consent

5.3 Withdrawal criteria

- Pregnancy (or desire for pregnancy) during the study
- Lack of compliance to any of the important study procedures in the discretion of the investigator
- Withdrawal on the participant’s request will be accepted at any time without further justification

Withdrawal does not affect the person’s statutory patient rights.

6. Screening

After having received a written and oral informed consent, participants will complete a screening visit that reviews the inclusion and exclusion criteria.

At the screening visit a urine sample will be analyzed for albumin/creatinine ratio and U-HCG (for women with childbearing potential). A blood sample will be analyzed for Hemoglobin A1c (HbA1c), hemoglobin, c-peptide, creatinine, eGFR, ALAT, potassium, sodium, TSH, cholesterol, triglycerides, VLDL, LDL and HDL. ECG, blood pressure, and heart rate will also be measured. Participants will also perform a Cardiopulmonary Exercise Test (CPET) to volitional exhaustion on a workload controlled cycle ergometer. If the participant has recently undertaken a CPET as part of their inclusion in the previous clinical trial at SDCC and reports having no overt changes to their habitual fitness routine, the data from this will be used. Please refer to appendix 1 for a thorough breakdown of both the CPET and moderate intensity exercise test protocols and procedures.

If the participant is eligible for the study, baseline characteristics will be obtained which include sex, age, race, diabetes duration, duration of insulin pump use, insulin pump settings, duration of Continuous Glucose Monitoring (CGM) use, CGM values from the previous 2 weeks, allergies, medical history incl. diabetes late complications, medications, educational status, occupational status, hypoglycemia awareness status, height, weight, physical activity status. All the data will be registered in the electronic case report form (eCRF) for each study participant.

If participants are not eligible to participate in the study, the results of abnormal tests, including positive pregnancy test, will be reported to the participant.

7. Randomization and blinding

This is a two period, crossover interventional study, in which there will be no randomization or blinding.

8. Pre-study procedures

Participants must refrain from alcohol consumption and strenuous physical exercise 24 hours before the study visit. Participants will also be instructed not to administrate insulin boluses 4 hours prior to the start of the study visit. Conservative administration of glucose tablets prior to study initiation will be allowed to avoid hypoglycemia.

Individuals will be instructed to avoid hypoglycemia (defined as a sensor value and/or finger prick value < 3.9 mmol/l) 24 hours before each study visit. Hypoglycemia will be avoided by frequently measuring their plasma glucose (PG) and by setting the CGM low alarm to 4.5 mmol/l. If the participant has a low alarm on the CGM, it should always be ensured by Blood Glucose Monitoring (BGM). CGM data for the previous 24 hours will be evaluated on each study day to check for episodes of hypoglycemia. If hypoglycemia (<3.0 mmol/l) has been present, the study visit is postponed for at least one day.

9. Study visit procedures

Arrival and preparation:

In-clinic phase:

- Participants will arrive at the research facility at Steno Diabetes Center Copenhagen in the morning following an overnight fast (incl. coffee, medicine and tobacco). Conservative administration of glucose tablets prior to study initiation will be allowed to avoid hypoglycemia.
- A urine HCG test will be performed for all female subjects with childbearing potential. If the test is positive, the primary investigator will inform the subject of the test result and the visit will be cancelled.
- A peripheral venous catheter will be inserted in the antecubital vein to draw blood.
- 15 mins before exercise, the target glucose level will be increased to 8.3 mmol/L in the AHCL system until 15 mins after end of exercise.
- Venous blood glucose sampling will be taken in 15-min intervals from -45 to -15 mins, 5-min intervals from -15 to +60 mins and 15-min intervals from +60 to +105 mins. Samples will be analyzed immediately to determine blood glucose concentrations and later to characterize the metabolomic, hormonal and physiological responses.

Intervention and exercise:

- Immediately before exercise start (t = 0), 150 µg s.c. glucagon will be administrated in the abdominal area at visit B No glucagon will be given at visit C.
- Subsequently, participants will start exercising on an ergocycle with moderate intensity (60% of VO_2-max, which is available from the CPET) for 45 mins.
- If the participant experiences hypoglycemia (PG < 3.9 mmol/l) during the exercise session, exercise will be stopped, and 15 g of oral glucose will be provided. The participant will then rest for the remaining time of the exercise period, and afterwards move on to the post-exercise observation period. If the PG has not risen to > 3.9 mmol/l after 15 min, another 15 g of oral glucose will be provided. In the unlikely event that the oral glucose consumption should not prove sufficient to

establish euglycemia, an intravenous glucose infusion will be provided. In both cases, the nadir PG level will be carried forward to the end of the observation period.

Post-exercise observation:

- After ending the exercise session, PG will be monitored for another 60 mins before concluding the study visit.
- The participants will consume a standardized low-glycemic index, carbohydrate-based meal (0.75 g carbohydrates per kg body weight) with their usual meal-time insulin dose.
- Following consumption of this meal, participants will be discharged from the laboratory.

The study visit will be stopped in the following circumstances:

- Inability to perform the exercise session.
- Cardiac discomfort.
- Onset of angina or angina-like symptoms.
- Shortness of breath, wheezing, leg cramps, or claudication.
- Signs of poor perfusion: light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold
- and clammy skin.
- Failure of the testing equipment.

Please see figure 2 illustrating the details for visit B

Trial day schematic – 2.5 hr. Laboratory phase

Phase	Pre-exercise (rest)	Exercise	Post-exercise (rest)
	Lab arrival; T= -45 min; T= -30 min; T= -15 min; T= 0 min	MICE ~ 60 % VO2max → 45 mins	T= +45 min; T= +60 min; T= +75 min; T= +105 min; Lab departure
Blood sampling*	T= -45, -30, -15, 0 min	Every 5 min	T= +45, +60, +75, +105 min
Glucagon (s.c. 150 µg)	-	T=0	-
SG target to 8.3	T=-15	-	T=+60**

Figure 1: Visit B, *PG, lactate and ketone bodies, metabolomics and hormones; ** the sensor glucose (SG) target will return to the initial value. MICE: Moderate intensity continuous exercise. Subcutaneous glucagon injection pertains to visit B only.

10. Study devices

The AHCL system used in this study is the Minimed 780G pump with the Guardian 3 or Guardian 4 sensor and receiver.

10.2 Minimed 780G insulin pump information

The MiniMed 780G system (Medtronic Diabetes, Northridge, CA) is a CE-marked AHCL system manufactured by Medtronic (16). The MiniMed 780G system is an automated insulin delivery system continuously anticipates insulin needs, adjust insulin delivery and correct high glucose levels automatically while helping to protect the user form lows glucose levels as well.

10.1 Guardian 3 and Guardian 4 sensor and receiver

Guardian 3 (17) and Guardian 4 are CE-marked products commercially available by prescription, indicated for patients with T1D. The sensors are both approved for non-adjunctive use, meaning that they can be used to make daily diabetes treatment decisions. The difference between them is that Guardian 4 does not require any calibration during the use, whether Guardian 3 requires calibration 2-3 times daily with a finger prick glucose measurement. The sensor data are stored locally in the pump without internet connection. Data from the pump will be uploaded to the local research server and to the participant's eCRF.

11. Study medication

11.1. Glucagon, GlucaGen®, Novo Nordisk, ATC code H04AA01

Please refer to the medication summary attached, appendix 2 for further details including adverse reaction profile (section 4.8).

In this study, the native glucagon powder (GlucaGen®, Novo Nordisk, Bagsværd, Denmark) will be used and will be dissolve 10 mins before administration.

The product is commercially available by prescription and indicated for participants with T1D. The recommended glucagon dose for severe hypoglycemia is 1 mg administered as a subcutaneous or intramuscular bolus. The administration may be repeated once if lack of clinical response. In this study, glucagon is delivered as a single dose of 150 µg. The total daily glucagon dose will therefore not exceed 1.0 mg per day.

The effects of glucagon are not expected to extend beyond the course of the actual study days. Accordingly, no post-study follow-up is planned, but participants are encouraged to contact the sponsor if they experience or suspect any adverse events to the study medication in the 24 hours following discharge from the research unit. This also applies to participants who discontinue study participation.

Glucagon is ordered via the hospital's pharmaceutical service associated with the pharmacy of the Capital Region. Batch number, date of expiration and the amount used to fill the pump is noted in the participant´s eCRF, see section 18 (Source data).

12.Endpoints

12.1 Primary endpoint

- Difference in percentage of time in target glucose range (PG: 3.9 - 10.0 mmol/l) during and for 1-hour after dynamic physical exercise $_{(0\ \text{min to}\ +105\text{min})}$ between visits B and C.

12.2 Secondary endpoints

- Difference in incidence rate of hypoglycemic events (PG<3.9 mmol/l) $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in time (min) to hypoglycemia (PG<3.9 mmol/l) $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in percentage of time below target glucose range (PG<3.9 mmol/l) $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in percentage of time above target glucose range (PG>10.0 mmol/l) $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in incidence rate of hyperglycemia (PG>10.0 mmol/l) $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in nadir PG concentration $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in peak PG concentration $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in incremental peak PG concentration $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in mean PG concentration $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in PG Area Under the Curve (AUC) $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in standard deviation in PG concentrations $_{(0\ min\ to\ +105min)}$ between visits B and C
- Difference in coefficient of variation in PG concentrations $_{(0\ min\ to\ +105min)}$ between visits B and C

13.Statistical considerations

To our knowledge, this is the first study to evaluate the glucose effects of s.c. injection of glucagon around exercise in individuals with AHCL-treated T1D. This is a proof of concept study to assess the use of low-dose glucagon in persons type 1 diabetes performing exercise during treatment with a hybrid closed-loop system with a control arm as a comparator on a sub-group of the full participant cohort. As such, no power calculation has been performed. The sample size of 16 participants has been chosen from a feasibility perspective. It is anticipated that at least 10 of whom will proceed to complete the control arm for comparative purposes in this proof-of concept study.

14. Safety and risk assessment

If PG drops below 3.9 mmol/l or the participant experiences unbearable symptoms of hypoglycaemia, 15 g of oral glucose will be administered, and subsequent close monitoring of PG will be ensured. The investigator can, at any given time, choose to discontinue the study visit if deemed necessary for safety reasons. Likewise, individuals can withdraw from the study at any given time.

In general, exercise is associated with a risk of hypoglycemia for people with T1D. In this study, some participants might experience an episode of mild hypoglycemia (PG level between 3.0 to 3.9 mmol/l). Symptoms of mild hypoglycemia (e.g. shakiness, dizziness, sweating, palpitations, hunger or headache) are relatively modest and previously experienced by all people with T1D. The risk of severe hypoglycemia during the study is very low, as the participants will be closely monitored with frequent glucose measurements and continuous clinical evaluation. Moreover, an IV catheter is maintained throughout the study visit which will enable fast elevation of PG if needed.

Administration of glucagon carries a small risk of transitory side effects with symptoms such as nausea and headache being the most frequent. However, as dose sizes in this study are small, the frequency of these side effects is expected to be low and the intensity mild.

The amount of blood extracted during the study visit equals about 250 ml (a regular blood donation amounts to approximately 500 ml), which is not expected to cause any adverse effects.

Performing venipuncture may inflict a short pain and a risk of a small hematoma, and there is a minimal risk of infection at the puncture site.

With regards to all other planned study procedures, the risk of complications or adverse events is negligible and certainly outweighed by the benefits of this study.

A study investigator, who also is a medical doctor, will always be present at the research facility during conduct of the study.

15. Adverse event management

Definition of adverse event (AE):

Any untoward medical occurrence in a patient or clinical investigation subject administering/using a product (which does not necessarily have to have a causal relationship with this treatment). An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether considered related to the product. Hyperglycemia and hypoglycemia handled by the safety procedures are not considered as AE.

Definition of serious adverse event (SAE):

An adverse event that results in death, is life threatening (an event in which the patient was at risk of death at the time of the event; not an event which hypothetically might have caused death if it was more severe), requires inpatient hospitalization or results in prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect.

Definition of serious adverse reaction (SAR):

A SAE for which a causal relationship to the study medication is at least possible (i.e. a causal relationship is conceivable and cannot be dismissed).

Definition of suspected unexpected serious adverse reaction (SUSAR):

A SAE which is unexpected and regarded as possibly or probably related to the trial/study product by the investigator. To evaluate whether the adverse reaction is to be expected, investigator shall refer to section 4.8 in the medication summary (Appendix 2).

Classification of intensity of AE:

Mild: No or transient symptoms, no interference with the patient's daily activities.
Moderate: Marked symptoms, moderate interference with the patient's daily activities.
Severe: Considerable interference with the patient's daily activities, which the patient find unacceptable.

Classification of causality of an AE:

Related: A causal relationship is conceivable and cannot be dismissed.
Not related: The event is most likely related to etiology other than the trial product

Final outcome classification of an AE:

Recovered/resolved: The patient has fully recovered, or the condition has returned to the level observed at trial entry.
Recovering/resolving: The condition is improving, and the patient is expected to recover from the AE.
Recovered/resolved with sequelae: The patient has recovered from the condition, but with lasting effect due to a disease, injury, treatment or procedure.
Not recovered/not resolved: The condition of the patient has not improved and the symptoms are unchanged, or the outcome is not known.
Fatal: This term is only applicable if the patient died due to the AE.
Unknown: This term is only applicable if the patient is lost to follow-up.

Investigations and potential treatment of an AE:

Dependent on the investigator's evaluation. Participants experiencing AEs will be monitored until satisfactory stabilization and/or full recovery.

Reporting of AE, SAE, SAR and SUSAR:

All events meeting the definition of an AE will be collected and reported. The causality is evaluated by the investigator. AEs will be collected from the beginning of the study day (visit B) until 12 hours after initial dosing of glucagon as the effects of glucagon are not expected to extend beyond this time frame. SAEs, SARs will initially be reported to the sponsor within 24 hours after the investigator has become aware of the adverse event. All follow-up data will be described in detail in a SAE-formula and subsequently given to the sponsor within 24 hours of obtaining the new knowledge. Previous non-serious AEs that becomes SAEs will follow the reporting of SAEs. It is the sponsor who will evaluate if the reported SAE and SAR are a SUSAR.

Sponsor will ensure that all information on SUSARs that are fatal or life-threatening is recorded and reported to the Danish Health and Medicines Authority (web-based form) as soon as possible and no later than seven days after the sponsor becomes aware of such possible side effects. Within eight days after reporting, sponsor will provide the Danish Health and Medicines Authority with all relevant information about the sponsor's and investigator's response to the alert and consequences for the further course of the study conduct.

All other SUSARs will be reported to the Danish Health and Medicines Authority within 15 calendar days after sponsor becomes aware of them. Moreover, a safety report will be compiled each year, which will be sent to the Regional Scientific Ethics Committee and Danish Medicines Agency.

Reporting at the end of the study:

All AEs and SAEs will be included and uploaded in the results section of the study EudraCT (https://eudract.ema.europa.eu/). The link to this report will be sent to the Danish Health and Medicines Authority and the Regional Scientific Ethics Committee within 12 months after the last participant has completed the project. SARs are reported to the Danish Health and Medicines Authority and the Regional Ethics Committee annually.

16. Extraction of biological samples

A research biobank will be established for all blood samples that are collected from the HYPO-AVOID study. At each sampling timepoint there will be drawn approximately 5,8 mL of blood; 0.5 mL will be disposed as waste, 0,3 mL for quantification of plasma glucose and lactate and 5 ml for biobanking. In total 250 mL blood will be drawn. All blood samples are designated with a code that cannot be directly referred to

the individual. Only PG, lactate and ketone bodies are analyzed immediately. The research unit has several freezers that are only used to store samples from different projects. The freezers are locked and can only be accessed by staff members from the research unit. Furthermore, the freezers are continuously monitored and alert a responsible for any significant temperature changes. The frozen blood samples will be analysed for metabolomic and hormones within 10 year after the study end, i.e. before 01/01/2032. If there is any remaining material left, the samples will be stored for future analyses after approval from the Capital Region's Videnscenter for Dataanmeldelser. If remaining material will be used for future studies, we will apply for approval by the Regional Scientific Ethical Committee.

The analyses will be performed at the SDCC CoreLab, Steno Diabetes Center Copenhagen and at Department of Biomedical Sciences, Endocrinology Research Section, University of Copenhagen.

17. Use of data from patient health records

Data from patient health records will be used during the screening process. By signing the informed consent, the participant grants access to information in the medical health record relevant to participation in the study. The access applies for the primary study investigator, sponsor (and representatives) and potential regulatory authorities (for quality control). After receiving the informed consent, relevant information from the medical health records will be revisited during the screening visit.

18. Source data

A separate electronic case report form (eCRF, REDCap) will be prepared for each study participant. Patient information will be retrieved from routine-care hospital databases (Sundhedsplatformen and Carelink). Information on diagnosis, age, sex, diabetes duration, insulin pump settings, medical history, medications, blood test results and ECG will be collected.

Glucose measurements are documented by the source files generated from the respective apparatus (YSI measurements from Yellow Spring Instruments 2900 STAT Plus). Data from devices without long-term internal memory (e.g. blood pressure apparatus) will be transferred directly to the eCRF.

A monitoring plan will be established in collaboration with the GCP-unit at Copenhagen University Hospital and includes full monitoring of informed consent, serious adverse events, inclusion and exclusion criteria. Source data will also be verified by the GCP-unit according to the monitoring plan.

19. Handling of personal data

The study will be carried out in accordance with the Helsinki Declaration after approval by the Regional Scientific Ethical Committee, Region H's Data Protection Unit (Videnscenter for Dataanmeldelser) and the Danish Medicines Agency.

All information on study subjects is protected according to law on processing of personal data and the law of health.

All health-related matters and sensitive personal data will be handled in accordance with the data protection act (databeskyttelsesloven) and GDPR (databeskyttelsesforordningen).

The electronic study database (REDCap) is password protected and located on the hospital network server which is continuously backed up. The electronic file with the key linking the CPR-number and all other information that is personally identifiable will be kept on a locked drive created for that purpose only. Only the study sponsor and investigators will have access to the study database.

All health-related matters and sensitive personal data (CRF, blood test result etc.) will be depersonalized. All participants will be given a number referring to their personal information, which will be stored securely and separately. Adequate anonymization of all personal data during data processing and publication will be ensured.

Data will be stored in coded form in 10 years after last participant last visit (LPLV) according to recommendations from the Data Protection Agency.

Study data may be shared with cooperating partners outside Steno Diabetes Center Copenhagen, but only in a form in which all personally identifiable information has been removed. Data will only be shared with cooperating partners outside SDCC after acceptance from "Videnscenter for Dataanmeldelser".

20. Time schedule

First participant first visit (FPFV) is expected in June 2022, while last participant last visit (LPLV) is expected in Marts 2024. Data analysis will be completed within 12 months after LPLV.

21. Project economy

The study is initiated by the investigators. All investigators are employed at Steno Diabetes Center Copenhagen or University of Swansea. None of the investigators have personal financial interest in the conduct or the outcome of the project.

The primary investigator, Sissel Lundemose, is not restricted by any means and no external parties hold rights to the study results. If further funding is achieved, the national ethics committee will be notified and the protocol, the protocol resume and the participant information documents will be updated.

22. Remuneration

Participants do not receive remuneration for study participation.

23. Insurance

Participants are covered by the state compensation scheme ('Patienterstatningsordningen' og 'Ordningen om erstatning for lægemiddelskader').

24. Dissemination of study results

Data will be processed and merged into at least one scientific article and published in an international peer-reviewed scientific journal. Positive, negative and inconclusive results will be published as soon as scientifically justifiable. The latest study protocol will be published at www.clinicaltrials.gov.

25. Ethical considerations

The risk of side effects when participating in this study is expected to be modest as elaborated on in the section 14 (Safety and risk assessment). A number of studies have previously demonstrated safety and efficacy of the low-dose glucagon for preventing and treating hypoglycemia, and glucagon has been well tolerated, safe and effective in all previous studies.

The study will be carried out in accordance with the Declaration of Helsinki and the principles of good clinical practice after approval by the Regional Scientific Ethics Committee and the Danish Medicines Agency. The study will be registered at www.clinicaltrials.gov. The GCP Unit, Copenhagen University Hospital, is responsible for study monitoring which includes full monitoring of informed consent, power of attorney and SAEs. Further, the participants agree to allow direct access to their source data/documents, including patient journals, during monitoring, auditing and/or inspection by an ethics committee, the Danish

Medicines Agency or any other countries' health authorities. Procedures for quality assurance and quality control will be applied according to local guidelines.

We expect that this study will provide important information on the use of low-dose of glucagon for prevention of exercise-induced hypoglycemia

Novelty and importance of this work
The proposed study investigating the optimal use of AHCL and the use of low-dose glucagon around exercise in people with T1D will provide clinically relevant, evidence-based information that will benefit both patients and practitioners. The study is a central step on the way to providing people with T1D with new management strategies for preventing and treating exercise-induced hypoglycemia. Hence, the investigators are confident that the possible risks and side effects for the participating subjects are outweighed by the expected benefits from the conduct of this study.

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29. Appendices

Appendix 1: Cardiopulmonary Exercise Test and Moderate intensity continuous exercise testing procedures

Appendix 2: GlucaGen Product Summary