DUAL-HORMONE CLOSED-LOOP GLUCOSE CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES

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Dual-Hormone Closed-Loop Glucose Control in Adolescents with Type 1 Diabetes

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The sponsor of **Dual-Hormone Closed-Loop Glucose Control in Adolescents with Type 1 Diabetes**, Kirsten Nørgaard, guarantees that the study will be conducted in accordance with this protocol and current legislation and regulatory requirements.

Contents

Abbreviations

AE	Adverse Event
CGM	Continuous Glucose Monitoring
CHO	Carbohydrate
Closed-loop control	Automated glucose control
CRF	Case Report Form
DTU	Danmarks Tekniske Universitet / Technical University of Denmark
HbA1c	Hemoglobin A1c
LBGI	Low Blood Glucose Index
MARD	Mean Absolute Relative Difference
MPC	Model Predictive Control
Open-loop control	Participant-controlled glucose control
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
SMBG	Self-Monitoring of Blood Glucose
SOP	Standardized Operating Procedures
T1D	Type 1 Diabetes
VAS	Visual Analog Scale
YSI	Yellow Spring Instruments 2900 STAT Plus

1. Background

People living with type 1 diabetes (T1D) need lifelong supply of external insulin to survive. They are advised to aim for near-normal blood glucose levels through intensive insulin therapy to optimize physiological and psychological well-being and reduce the risk of chronic diabetes complications[1,2]. Unfortunately, this therapy approach is associated with increased disease burden, risk of hypoglycemia and weight gain[3]. Hypoglycemia is caused by insulin overdosing in combination with a defective counter-regulatory response[4]. If untreated, it can cause seizures, unconsciousness or death[5]. Non-severe hypoglycemia, particularly nocturnal, is very frequent with longer duration in children and adolescents than in adults[6]. Around 75% of hypoglycemia seizures in children and adolescents happen at night-time and fear of these episodes is a major source of stress and anxiety for families and caregivers of people with T1D[7]. Furthermore, fear of hypoglycemia is the main limiting factor in diabetes management as it keeps people with T1D from optimal insulin dosing[8,9].

The obesity rate for people with T1D has shown to surpass that of the general population and around 42-48% of the adolescents with T1D are either overweight or obese[10–14]. Besides the intensified insulin usage, weight gain may be further increased by the frequent oral carbohydrate intakes needed for treating hypoglycemic events. If people with T1D do not take these extra calories into account by reducing size of their main meals, they will inevitably gain weight. Moreover, people with T1D perform less exercise than the general population due to fear of hypoglycemia that additionally increases the risk of overweight and cardiovascular disease[15–17].

Intensive insulin therapy is administered by multiple daily pen injections of insulin or by insulin pumps. Blood glucose levels can be evaluated by finger stick measurements (referred to as self-monitoring of blood glucose, SMBG) or by glucose sensors (referred to as continuous glucose monitoring, CGM). The CGMs can either be used as stand-alone or integrated with insulin pumps. Some sensor-integrated insulin pumps can automatically stop insulin infusion when CGM levels are low or trending toward hypoglycemic levels. Adding CGM to insulin therapy significantly improves HbA1c and decreases hypoglycemia[18,19]. However, only a minority achieves optimal glycemic goals (20%), avoids hypoglycemia and overweight with use of these recently advanced diabetes technologies[12,20–23].

The most advanced commercially available technology for T1D is the hybrid insulin-only artificial pancreas system. Such systems can automatically stop and even increase insulin delivery to maintain CGM levels within near-normal glucose range. Still, the users must enter the ingested carbohydrate amount into their pump to receive insulin boluses for their meals. These systems ease the burden of constant treatment decision-making and at the same time it safely intensifies insulin therapy such that more people with T1D reach treatment goals. However, hypoglycemia and weight gain are still present.

Most artificial pancreas systems used for research consist of an insulin pump, a CGM, and a control algorithm residing on a mobile computer, e.g. smartphone that continuously (every 5-15 min) computes the optimal insulin dosage from the CGM values. Despite all of this, there is still an unmet medical need to reduce the risk of hypoglycemia, weight gain and diabetes burden.

We hypothesize that the addition of glucagon to an insulin-only artificial pancreas system may improve glucose control and reduce the risk of hypoglycemia, weight gain and diabetes stress[24].

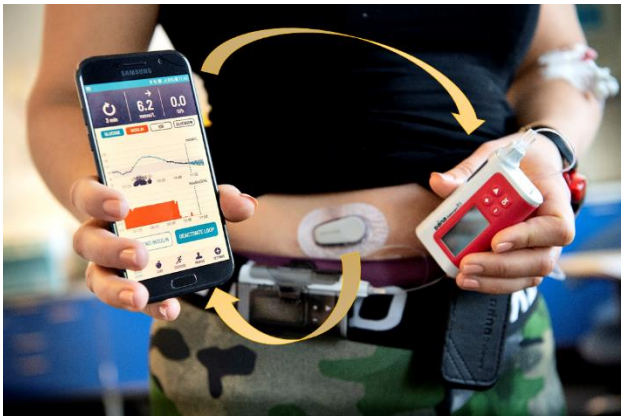

Figure 1: Closed-loop glucose control system

1.1. DiaCon Closed-loop approach

In 2008, our research group started a collaboration with the Technical University of Denmark (DTU), called DiaCon (Diabetes Control). We developed a control algorithm based on Model Predictive Control (MPC) theory, and by using insulin pumps and CGMs we conducted our first clinical study in 2011-2012 that demonstrated that our insulin-only artificial pancreas system is safe and effective for overnight blood glucose control[25]. For daytime blood glucose control, however, we believe that the system needs to be further advanced due to glucose excursion related to physical activity and different meal composition[26]. Consequently, we changed our strategy from an insulin-only to an insulin-glucagon approach[24]. We have extended our system to include a second pump for glucagon delivery and correspondingly we have from 2012-2018 developed our smartphone application and the control algorithm to compute both insulin and glucagon dosages[27,28] (Figure 1).

Currently available glucagon formulations (GlucaGen from Novo Nordisk or Glucagon from Eli Lilly) require reconstitution of dry powder in aqueous solution immediately prior to each use. The process of reconstitution and delivery is complex and requires adequate education of families and caregivers. Furthermore, available glucagon formulations form fibrils rapidly after reconstitution, resulting in the product being unusable within 1 day of reconstitution. Thus, a glucagon formulation with longer stability would increase the feasibility of dual-hormonal pump delivery devices. Some pharmaceutical companies (Zealand Pharma, Eli Lilly and Xeris) have developed soluble and stable glucagon products that are undergoing Phase 3 trials. For the proposed clinical study, we will however use GlucaGen® from Novo Nordisk.

Four other research groups have published studies of dual-hormone closed-loop glucose control[29–32] with the use of GlucaGen®. The Boston, Oregon and Amsterdam groups practice alternating glucagon and insulin administration, whereas the Montreal group primarily uses glucagon as a safety feature. One of the main differences between these approaches is the aggressiveness of the insulin dosing. In our group, we practice conservative insulin dosing, while glucagon delivery is only considered a safety feature as seen for the Montreal group.

1.2. Preliminary data

In our ongoing 33-hours in-clinic study on adults, our dual-hormone algorithm performs well, i.e. it controls the blood glucose safely and effectively; the percentage of time in the hypoglycemic range is reduced compared with single-hormone control; and the time spent in the normoglycemic range is increased compared with single-hormone control, despite the system being challenged with an exercise session and meal insulin boluses that are both underdosed for breakfast and overdosed for dinner (Figure 2). Initially, there were issues regarding the algorithm and medical devices, e.g.

infusion set failures, occlusion of the glucagon infusion set, and disconnections of the smartphone to the sensor and the pumps. These issues have been solved accordingly and were less frequent in the last part of the adult study (Investigator's Brochure). The next step is to confirm that our dual-hormone artificial system can provide as promising results in adolescents as observed in adults.

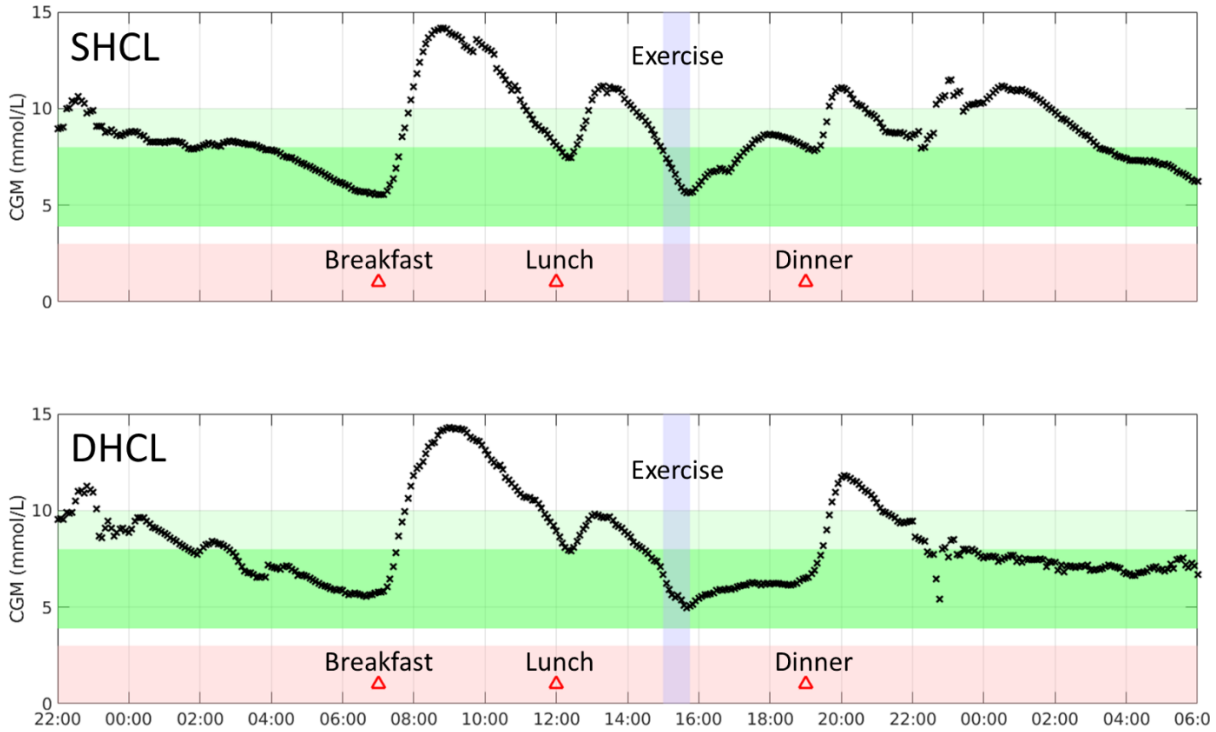

Figure 2: Preliminary results from the adult closed-loop study. Mean CGM values during dual hormone (DHCL) and single hormone closed-loop control (SHCL). DHCL had similar time in hypoglycaemia compared with SHCL (3.63% vs. 3.75%, P = 0.46) but with less carbohydrate rescues during the study (0.8 vs. 1.42, P = 0.04).

1.3. Rational for testing the system in adolescents

Adolescence is a critical period of growth accompanied by changes in interpersonal roles, responsibilities, and identity development. Not surprising, these years are more complex for adolescents diagnosed with type 1 diabetes. In addition to experiencing the same challenges as their healthy peers, adolescents with diabetes must contend with intensive medical regimens, regular clinic appointments, complicated carbohydrate calculations, and frequent monitoring of blood glucose levels. Although tools are currently available to facilitate more optimal glycemic control and decrease the risk of long-term, negative health consequences, adolescents with type 1 diabetes have the worst glycemic control compared with the rest of the T1D population[20,33]. We hypothesize that an artificial pancreas system may improve the glucose control in this population because the number of decision-makings (i.e. glucose monitoring and insulin dosing) are reduced. Even though our closed-loop system performs well in the adult population, we need to test whether the system can perform equally well in this age group with rapid changes in hormones and lifestyle. Pubertal status affects the glucose homeostasis by changing insulin sensitivity during all daily activities (rest, meal intake, exercise and sleep). The biggest differences in pubertal status occur around the age of 13-17 years. Therefore, we want to investigate whether our closed-loop system can meet and adapt to the huge variation in insulin sensitivity across different pubertal status, seen in age 13-17 years.

2. Hypothesis

We hypothesize that we have developed a safe and effective dual-hormone closed-loop system for adolescents with type 1 diabetes and that this system is superior to single-hormone closed-loop therapy.

3. Aims

The aims of this two-phase project are to 1) demonstrate proof-of-concept and 2) to compare dual-hormone with single-hormone closed-loop glucose control.

4. Study design

This study has two separate phases: a pilot and clinical phase.

4.1. Pilot Study

The pilot study tests the operability of our dual-hormone system in adolescents with T1D. Two to four 26-hour study visits with dual-hormone closed-loop glucose control will be performed. The study procedures are identical to the procedures described in Phase 2, except participants are only receiving dual-hormone therapy and only blood samples for glucose, lactate and ketones are drawn.

In this study phase, we expect to identify potential technical challenges that were not presented in the previous adult study. Furthermore, we will evaluate whether adjustments of the control algorithm for insulin and glucagon delivery are needed. We do not expect to perform more than two study visits because the system has been tested in the adults. However, depending on the number and character of the challenges from the first two phase 1 study visits, another one or two 26-hour study visits may be needed to evaluate the trouble-shootings. When the technical solution and the algorithm tuning are satisfactory, assessed by the research group, we will proceed to the next phase.

4.2. Clinical Study

A randomized, single-blinded, cross-over study of glycemic control during dual-hormone therapy compared with single-hormone therapy in adolescents with type 1 diabetes (figure 5). Participants are blinded to the intervention, i.e. whether they are receiving single- or dual-hormone therapy. The reason for blinding the participants to the method of glucose control is based on the assumption that knowledge about the method could affect stress hormone levels and thereby glucose homeostasis. All study-related procedures described below are common to phase 1 and phase 2.

5. Study subjects

5.1. Inclusion criteria

- Age = 13-17 years
- T1D duration ≥ 2 years

- Insulin pump therapy ≥ 1 year
- Using CGM or isCGM (Flash Libre)
- HbA1c ≤ 9.0% (75 mmol/mol)
- Using carbohydrate counting

5.2. Exclusion criteria

- Allergy to glucagon or lactose
- Allergy to faster insulin aspart (FiAsp)
- Pheochromocytoma
- Self-reported lack of hypoglycemia symptoms when blood glucose is < 3.0 mmol/l
- Inability to follow study procedures, e.g. exercise, sleeping, blood sampling, and meal intake
- Pregnancy, nursing, plan to become pregnant or sexually active and not using adequate contraceptive methods (intrauterine device, contraceptive pill, patch or injection)
- Use of anti-diabetic medicine (other than insulin), corticosteroids or other drugs affecting glucose metabolism during or within 30 days prior to study participation
- Other concomitant medical or psychological condition that according to the investigator's assessment makes the participant unsuitable for study participation

5.3. Withdrawal criteria

- Lack of compliance to any of the important study procedures in the discretion of the investigator
- Unacceptable adverse effects in the discretion of the investigator
- In case of pregnancy (or desire for pregnancy), female subjects are withdrawn

Withdrawal on participants request will be accepted at any time without further justification. Participants who complete or withdraw from the study continue their usual follow-up visits at the diabetes clinic. Withdrawal does not affect their statutory patient rights.

5.4. Recruitment and informed consent procedure

Participants are recruited from the outpatient clinic at Herlev University Hospital. Potential eligible participants are identified by information about diagnosis, diabetes duration, age, sex, insulin treatment modality, HbA1c, comorbidities, and allergies that are given from the treatment responsible personnel to the primary investigator or other personnel involved in the recruitment procedure. If potentially eligible, the treatment responsible health care personnel will ask the patient and the parents at the upcoming visit whether they are interested in participation. A written participant information will be given along with the brochure: "Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt". Identified participants who do not have an appointment at the clinic in near future, will be contacted by a letter or by a secured e-mail (E-boks) with the written information.

Occasionally, we are contacted by patients who want to participate in our studies although their regular diabetes therapy is managed at other hospitals. These persons can also be recruited for this study if they meet the eligibility criteria listed above.

For patients interested in study participation, a subsequent consultation will be scheduled and spoken information will be given by the recruiting investigator in a quiet environment. The recruiting investigators will either normally work or have worked at the paediatric department, having the knowledge to consult with adolescents at different age and with their parents. They will ensure that the patients and their parents are adequately informed about the study rationale and design, in written and spoken words. The information to the participants will be delivered in accordance with their age and maturity. The study personnel have experience in handling this special age group in research and clinic and are therefore taking good care of clear communication for the exact age group and in informing the participants and caregivers as well. The participant and the parents will also be informed that personal data concerning study participants may be disclosed to the Danish Health and Medicines Authority, health authorities of other countries, the Regional Scientific Ethics Committee and the GCP Unit, Copenhagen University Hospital, as part of the agencies' monitoring activities. The participant and their parents will have the opportunity to ask questions and bring a companion to the interview. Consent will be mandatory to be received oral from the participant itself, written from both caregivers with parent authorities if participants is under 18 years. Patients between 15-17 years will give written consent on their own for participation together with the written consent from the parent authorities. If the participant during the study turns 18 years old, a renewed informed consent will be requested from the participants and not from their parents. Before signing the consent form, the participant and their parents are given a minimum of 24 hours and maximum of seven days to re-consider. Should they need further time, a follow-up meeting will be scheduled. Participant and parents are informed that they may, at any time, withdraw their informed consent for participation in the study without these having consequences for the future treatment. The informed consent procedure described above may be scheduled at the same day as the screening visit; however, no study-related examinations will be conducted before the informed consent form has been signed by the parents.

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

If a participant and/or their parents want additional information about the study before or after giving consent, the sponsor and the clinical investigators can be contacted. Information details are given in the study information letter.

6. Study procedures

For all study visits, one of their parents or their legal guardian will be present. However, participants above 15 years of age can stay without, if they wish to do so.

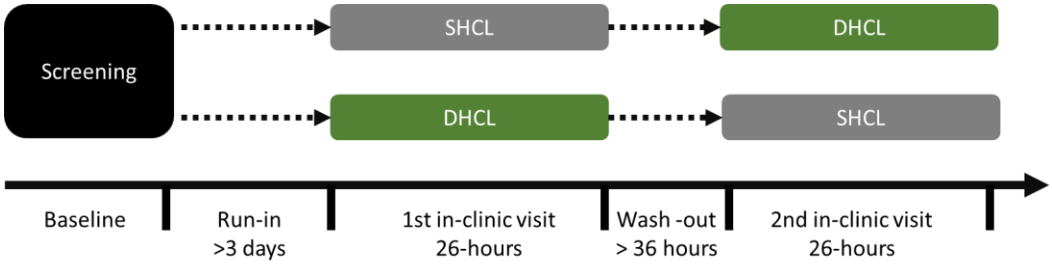

Figure 3: Study design. SHCL: Single Hormone Closed-loop with Fiasp and Dummy pumps. DHCL: Dual Hormone Closed-loop with Fiasp and Glucagon pumps.

6.1. Pre-study

Screening visit: At the screening visit inclusion and exclusion criteria will be reviewed. The following data will be recorded: sex, age, race, pubertal stage (Tanner), diabetes duration, duration of insulin pump use, insulin pump settings (basal rates, insulin sensitivities, insulin:CHO ratios, insulin action time), total daily insulin dose (average of previous fourteen days), CGM usage, allergies, medical history, medications, height, weight, blood pressure, pulse rate, and hemoglobin A1c (HbA1c).

Randomization: The order of the two study periods will be determined by block randomization (using random blocks of 2 and 4). The randomization will be performed using the electronic capture system, REDCap. An allocation table generated by sealedenvelope.com will be uploaded to REDCap by a person not otherwise involved in the study. Using the randomization functionality of REDCap, the subjects will be randomized after ensuring that all criteria for participation have been met. The study is single-blinded and only the participants and their parents will be blinded to the intervention. Preparation, allocation and administration of the study drugs will be controlled and verified by two persons.

Continuous Glucose Monitoring Initiation: Two days before study start, one DexCom G6 CGM is inserted into the abdominal subcutaneous tissue of the study subject. Study subjects or their parents who are familiar with the procedure can do the insertion at home. However, the participants have to start the sensor up with the Dexcom receiver and not with the Dexcom app, since the transmitter only can be paired with one app. The pairing to our app is essential to run our closed-loop system. Participants who are inexperienced with the Dexcom CGM will get the sensor inserted at the research facility. No calibrations are needed for these CGMs.

Participant preparation: Prior to the first in-clinic study day, insulin pump settings (basal rate, insulin action time, insulin sensitivity factor, insulin:CHO ratio) are recorded as well as the insulin and CGM profiles for 3 days. The data are used for setting up the control algorithm.

Further, 24 hours before study start, the participant refrains from alcohol consumption and strenuous physical exercise. Participants are requested to consume a low-fat evening meal with 50% energy

from carbohydrates and covering a maximum of 40% of daily requirements adjusted to age and weight no later than 15:00 on the day of the study start. Participants have to consume the identical food before the second visit. Food is not delivered to the participants for this purpose.

6.2. Study visit

Upon arrival at the research facility at Steno Diabetes Center Copenhagen, the participant's own pump and CGM are disconnected, and the two study pumps are attached. For dual-hormone control, the study pumps will be filled with FiAsp® and GlucaGen®. For single-hormone control, the study pumps have been filled with FiAsp® and isotonic saline. Pump filling is conducted before the participant arrives at the research facility. The glucagon/saline pump and the insulin pump are clearly marked and cannot be confused. The participants are blinded to the treatment, as isotonic saline by its looks is indistinguishable from GlucaGen®. The placebo pump ('dummy' pump) will not infuse the saline and cannot be detected by the participant. The investigator will at predefined time points access the pumps and manage the pumps according to standard operation procedures (SOP_worksheet).

In addition, participants are provided with a blinded wearable activity and sleep monitoring device (ActiGraph GT9X Link, Pensacola, FL) for activity level estimation and sleep assessment during study participation, figure 4. The Actigraph is connected to a Bluetooth® Polar heart rate monitors during the exercise session.

Figure 4: Actigraph® worn on wrist for monitoring activity and sleep.

Female participants deliver a urine sample for pregnancy testing. A sampling cannula is placed in an antecubital vein.

The CGM will be calibrated with fingerpick glucose meter (Contour next®, Ascensia Diabetes Care) before initiating the closed-loop control. Even though it is not needed to calibrate the sensor, the accuracy of the sensor is better after one calibration.

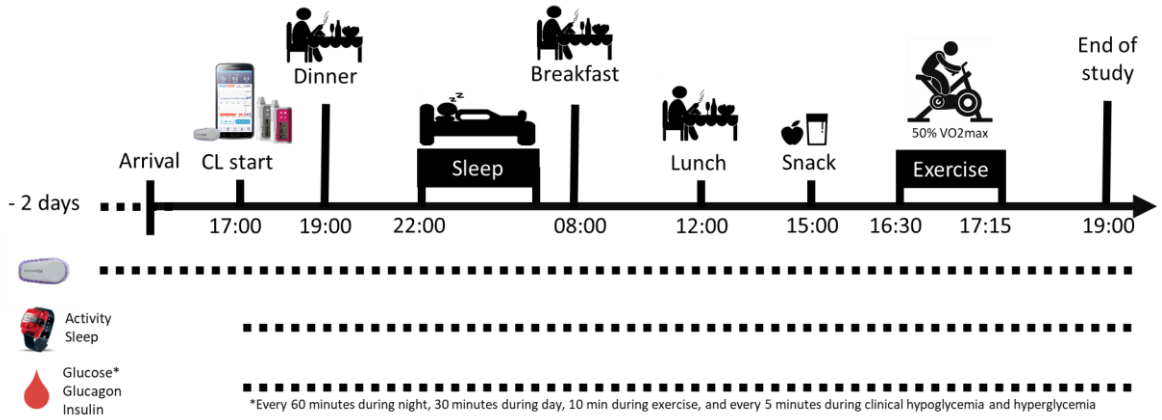

Figure 5: Study flow chart

At day 1 17:00 the study is initiated, and the closed-loop system takes over glucose control. Except from the control approach (single- vs. dual-hormone), the study days are identical and as depicted in Figure 5. During the study visit, participants can move around freely, but they can only perform actual exercise during the stationary bike exercise session at day 2 16:30. At nighttime, they are encouraged to be in bed and, if possible, sleep. At predefined timepoints, we measure blood pressure and pulse, and we ask them to rate their nausea level on a visual analog scale (VAS) from 1 to 100 to assess possible side effects of glucagon (vomiting rates as 100). In addition, we ask them to self-rate blood glucose levels before bedtime (23:00), upon wake-up (07:00-08:00), before each meal, before exercise, and every 10 minutes during exercise (SOP_worksheet).

Participants are asked to assess the amount of carbohydrate in each of the meals and snack served during the study. These procedures are performed when participants are resting.

Single-hormone and dual-hormone closed-loop control study sessions end 26 hours after study start. The study pumps and the CGMs are disconnected and the participants reconnect their own pumps and CGMs.

The two study sessions are separated by at least 36 hours.

6.2.1 Meals

Participants will be served with standardized meals according to their normal carbohydrate intake assessed by their insulin pump data for the previous 7 days. Participants are instructed to count and announce all carb intakes for these 7 days.

According to their daily carbohydrate intake the following will be given at the study visits:

Meal content:

- Intake of <100 g per day: 30 g CHO for each meal
- Intake of 100-150 g per day: 50 g CHO for each meal
- Intake of 150-200 g per day: 60 g CHO for each meal
- Intake of >200 g per day: 70 g CHO for each meal

Snack content:

- 1/3 of the CHO amount for each meal is given, e.g. 30 g for meals equals 10 g for snack.

The hospital kitchen will prepare the meals for breakfast, lunch and snacks, while the dinner is provided by a prespecified meal from MacDonald's.

Meal insulin bolus is administered by the control algorithm after the meal has been announced. The insulin needed to cover the CHO content of a meal is calculated by the algorithm and depends on the amount of CHO announced to the algorithm. Participants are not informed about the CHO content of the meal or the size of the meal dose. Before consuming each meal, they are asked to estimate the CHO content of the meal that will be used for announcement to the algorithm.

6.2.2. Exercise

Exercise is conducted at a stationary bike at moderate intensity defined as 50% of the heart rate reserve calculated using the formula of Tanaka et al.[34] ($HR = 50\% ((208 - 0.7*age) - HR_{rest}) + HR_{rest}$). The total duration of pedaling is 45 min or until clinical hypoglycemia (<3.0 mmol/l). If plasma glucose is below 3.9 mmol/l within 30 minutes before the exercise session, a 15 g dextrose is given orally every 15 minutes till glucose is above 3.9 mmol/l. These dextrose supplement will be accounted as a carbohydrate rescue in the analysis. Exercise session will start once the plasma glucose concentrations are above 3.9 mmol/l. However, subject will only bicycle for the remaining time of the exercise timeslot from 4.30 PM to 5.15 PM, i.e. if participants starts bicycling at 5:00 PM, they can exercise for 15 min or until clinical hypoglycemia (<3.0 mmol/l). Subjects will rate their exercise intensity using the Borg scale every 10 minutes.

6.2.3. Blood sampling procedures

A venous blood sample (0.5 ml) is collected every 10-30 min during closed-loop control and used for evaluation of CGM accuracy. We spin the sample and measure the plasma glucose value and the plasma lactate value using Yellow Spring Instruments 2900 STAT Plus (YSI). Immediately after the sample has been analyzed, we discard it. Additional measurements of plasma glucose and blood ketone (Freestyle Precision B-ketone, Abbott, Abbott Park, Illinois) may be performed at any time point if considered appropriate by the attending physician and/or if the participant is hypoglycemic or hyperglycemic (SOP_Dysglycemia). The total amount of blood to be drawn per participant does not exceed 120ml. The exact amount of blood drawn depends on the time spent in hypoglycemia and hyperglycemia (SOP_Dysglycemia).

6.2.4. Glucose sensor procedures

Throughout the study day, sensor accuracy is evaluated every 30 minutes by comparing the sensor value with an YSI value. If the difference between a CGM value and a YSI value is more than 20%, the attending study personnel will assess whether to calibrate the sensor. Differences between CGM and YSI values are, however, expected two hours post-meal, post-snack or during exercise and in these periods, no calibrations will be performed even if the difference between CGM and YSI values exceeds the limits given above. If discrepancy persist even after calibration and restarting the sensors, the primary investigator will decide on whether to proceed or discontinue the study visit.

6.2.5. Procedures for device disconnections

CGM disconnection: If the CGM is disconnected, the application will immediately show red-coloured glucose values. If the CGM is disconnected for 15 min, an alarm will be raised from the smartphone and the attending study personnel will troubleshoot the system.

Pump disconnection: If the insulin and/or the glucagon pump do not respond to the smartphone for 15 min, an alarm will be raised from the smartphone and the attending study personnel will troubleshoot the system.

Smartphone disconnection: If the smartphone application crashes (an unexpected error forcing the application to stop), the application will automatically restart. An alarm will be raised, and a tool bar will appear on the button of the screen, indicating the app crash. The study personnel will immediately restart the system.

7. The Dual-Hormone Closed-Loop System: Medical Devices and Medication

The dual-hormone system consists of several components, i.e. two Dana-RS insulin pumps (one containing insulin and one containing glucagon or saline), one DexCom G6 CGM sensor, and one Samsung Galaxy A5 2017 Android phone operating the control algorithm for the closed-loop glucose control (Figure 6 and for more details see the Investigator's brochure).

Figure 6: Devices for our closed-loop system: 2 x Dana-RS insulin pump, 1 x DexCom G6 CGM, and Samsung Galaxy A5 2017 Android phone operating the control algorithm for the closed-loop glucose control.

7.1. Medical devices

7.1.1. Insulin Pump, Dana Diabecare RS® insulin pump

For insulin infusion we use a CE-marked Dana Diabecare RS insulin pump (SOOIL Development, Seoul, Korea). The product is commercially available by prescription and is indicated for insulin infusion in participants with T1D (see instruction for use). The same pump is used throughout the study, however, consumables including CE-marked cartridges and infusion sets (Dana Inset II,

Unomedical, ConvaTec) are discarded at the end of each study. Pump and infusion set are depicted in figure 1. All investigators are trained and experienced in the use of the Dana Diabecare RS insulin pump. For this study all device handling is managed by the investigators and accordingly no subject training is needed. The investigators will insert the pump according to manufacturer's instructions.

The pump has a mechanical dosing resolution of 0.01 unit. The reservoirs can hold 300 units (3 ml) of insulin. The pump will be slave to the control algorithm controlled by the Samsung Galaxy A5 2017 Android phone.

7.1.2. Glucagon Pump, Dana Diabecare RS® insulin pump

For glucagon infusion, we use the CE-marked Dana Diabecare RS insulin pump (SOOIL Development, Seoul, Korea). The same pump is used throughout the study, however, consumables including CE-marked cartridge and infusion set (Dana Inset II, Unomedical, ConvaTec) are discarded at the end of each study. Pump and infusion set are depicted in figure 1. All investigators are trained and experienced in the use of the Dana Diabecare RS insulin pump. For this study all device handling is managed by the investigators and accordingly no subject training is needed. The investigators will insert the pump according to manufacturer's instructions.

As mentioned above, the product is only CE-marked for insulin infusion and the indications do not include glucagon infusion (see instruction for use). However, we have performed a similar study on adults. Other dual-hormone studies have used GlucaGen® in other insulin pumps but with similar cartridge and infusion sets as we will use in this study. In the first part of the adult study, occlusion issues were present but after shifting to Unomedical infusion sets, we have not experienced any infusion set failure. None of the other studies using Unomedical infusion sets have reported malfunctions regarding the pump infusion of GlucaGen® [35–40].

On the placebo day, the pump will function as a "dummy" pump and will not infuse anything. The pump will therefore not be controlled by the control algorithm. However, the pump is filled with isotonic saline in order to uphold the blinding and to avoid alarms indicating empty cartridge from the pump. Still, the solution will not be infused to the participant.

7.1.3. Continuous Glucose Monitoring, DexCom G6®CGM

For glucose monitoring we use CE-marked DexCom G6 (DexCom, San Diego, California) CGMs (figure 7). The product is commercially available by prescription and indicated for participants with T1D (see instruction for use). All investigators are trained and experienced in the use of the DexCom G6 continuous glucose monitor. For this study all device handling is managed by the investigators and accordingly no subject training is needed. The investigators will insert the continuous glucose monitoring device according to manufacturer's instructions.

Figure 7: DexCom G6 continuous glucose monitoring system with (1) inserter, (2) sensor and transmitter, and (3) display device

7.1.4. Control algorithm

The control algorithm is not CE-marked. It is constructed by use of MPC theory and consists of two main components: an insulin controller and a glucagon controller. The system can operate with either the insulin controller alone or with both controllers activated simultaneously. When only the insulin controller component is activated, the system exerts single-hormone control. When both the insulin and the glucagon controller components are activated, the system exerts dual-hormone control. However, even with both controllers activated, the system does not deliver insulin and glucagon simultaneously. The MPC algorithm calculates the appropriate doses of insulin and glucagon to be delivered by the pumps (SOP_DiaCon_algorithm).

Software/algorithms developed by DiaCon are updated since the adult study and comprise the following:

7.1.5 Dexcom CGM communication:

DIACON_AP_ANDROID_CGM_DEXCOM VERSION 2.0

Dana RS insulin pump communication:
DIACON_AP_INSULIN_ANDROID_PUMP_DANARS_VERSION 2.0

Dana RS glucagon pump communication:
DIACON_AP_GLUCAGON_ANDROID_PUMP_DANARS_VERSION 2.0

Model Predictive Control Algorithms and Logics
DIACON_AP_MPC_CGM_INSULINGLUCAGON_PUMP VERSION 2.0

Grahical User Interface (GUI)
DIACON_AP_GUI_ANDROID VERSION 2.0

The software/algorithms developed by DiaCon will be archived and updates/modifications will be given a new version number. Each major version number will be associated with a major clinical trial. If minor modifications are made, they will be given a number such as VERSION 2.1 and a logbook will explain the changes made. All software used in clinical trials will be tested extensively by simulation in advance, go through a DiaCon review procedure, and be archived. The main changes introduced in version 2.0 increased the safety of the system. They are the following:

1) Basal rate upper limit: The maximum insulin delivery is set to 2.5 times the basal rate.

2) Boluses: Boluses are only allowed within 2 hours after a meal. The insulin bolus is limited to 1.3 times the bolus computed using the participant specific insulin-to-carbohydrate ratio (ICR). The total allowed dose is given by the formula: umax,bol =1.3 ∗ (gram carbohydrate content in meal) / ICR.

If a second meal is given within 2 hours after a meal, the timer is reset and the new maximum bolus is added to the current maximum bolus.

3) Glucagon: The glucagon is always administered as boluses. The glucagon limit has been raised from 200 to 300 µg over 2 hours. The glucagon threshold has been raised to 4.5 mmol/L instead of 4.0 mmol/L.

4) Pump: An alarm sounds from the smartphone if the system cannot connect to the pump.

5) Sensor: An alarm sounds from the smartphone if the system cannot connect to sensor.

7.1.6. Communication platform

The control algorithm resides on a CE-marked Samsung Galaxy A5 2017 Android phone (figure 8, #1). To secure data the Android phone do not have a SIM card and no Wi-Fi connection is allowed. All codes and commands are executed on the Android phone. The Android phone is connected to the Dexcom G6 CGM transmitter (figure 8, #2) via a Bluetooth Low Energy (BLE) connection and receives a new glucose value every 5 min. The glucose values serve as input to the controllers. The controller output, i.e. the insulin or glucagon dose to be delivered, is communicated from the Android phone to the pumps (figure 8, #3 and #4) via BLE. The Android phone handles all the BLE communications. Data are stored locally on the Android phone. After the trial the data are accessible for researchers by connecting a private computer located at the hospital to the Android phone using USB (figure 8, #5).

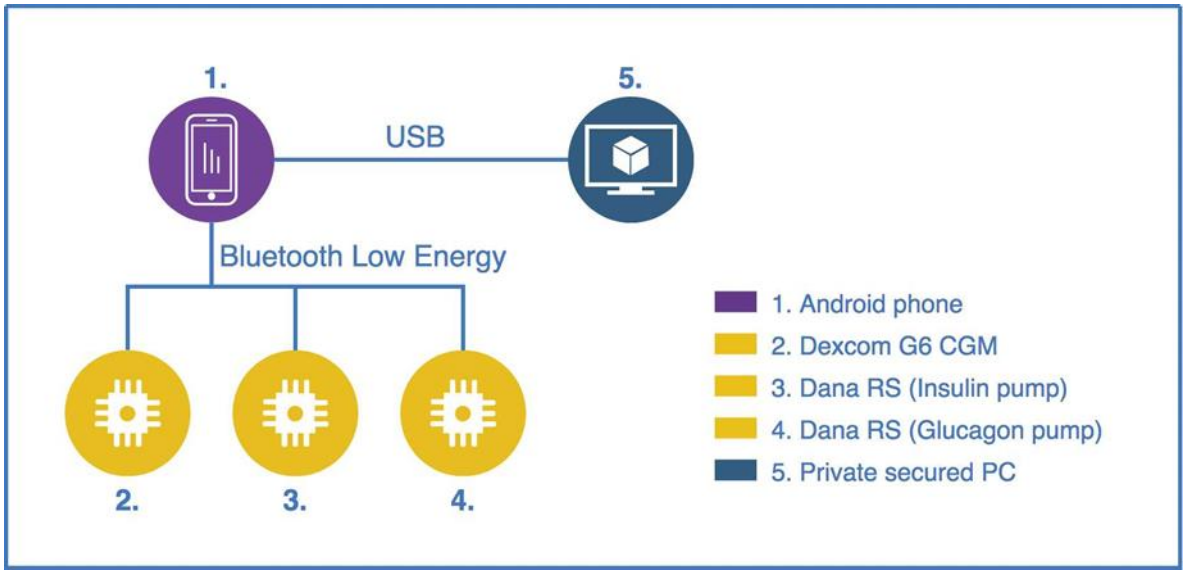

Figure 8: Deployment diagram of the complete set up of the closed-loop system

If the BLE connection between the communication platform (the CGM receiver) and the CGM is temporarily lost, i.e. no glucose value is transferred; insulin estimations will be based on the controller prediction. The attending physician evaluates safety of the insulin dosing and is always able to overrule the closed-loop system.

Our technical collaborators from DTU are responsible for training the investigators in the use of the communication platform prior to study start. A person from DTU will be present at the research lab during study phase 1 to assist in overcoming any start-up difficulties. During study phase 2, the person can be contacted by telephone if any difficulties arise. For this study all device handling is managed by the investigators and no subject training is needed.

In order to start the system, the two DanaRS pumps and the DexCom G6 transmitter should be paired to the Samsung Galaxy A5 2017, participant specific information (study id and controller variables) should be entered, and finally device connection and communication with controller unit should be verified. The investigators will be trained in this procedure before study start. For this study all device handling is managed by the investigators and accordingly no subject training is needed.

Any device deficiencies detected during the study will be reported to the manufacturers of the devices involved.

7.2. Study medication

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

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

The product is commercially available by prescription and indicated for participants with T1D. The recommended glucagon dose for children above 10 years old living and acutely suffering from 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 microboluses up to every five minutes and the dose size is based on the current blood glucose value. It is expected that in this study the total daily glucagon dose will not exceed 1.0 mg per day. The glucagon pump is filled with freshly reconstituted glucagon 1 mg/ml every 13 hours, i.e. before study start and at 14:00 (other groups have reported uncomplicated use of glucagon with reconstitution intervals of up to 24 hours).

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 eCRF.

7.2.2. Insulin, Fast-acting insulin aspart, FiAsp®, Novo Nordisk, ATC code A10AB05.

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

The product is commercially available by prescription and indicated for participants with T1D. Insulin is delivered as microboluses up to every five minutes and the dose size is based on the current blood glucose value. It is expected that the total daily insulin dose will be similar to the participant's usual daily dose. In general, the total daily dose is dependent on food intake, exercise and health status. The method of insulin delivery (microboluses vs. continuous infusion and automated versus participant-driven control) differs from current practice, however, otherwise participants receive insulin in accordance with usual standard of care, i.e. we try to achieve near-normalization of blood glucose values. The insulin pump is filled with fast-acting insulin aspart 100 IE/ml before study start.

Fast-acting insulin aspart 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 eCRF.

7.2.3. Saline (not a study medication)

On the single-hormone study days, a 'dummy' pump is filled with isotonic saltwater (sodium chloride 9 mg/dl). However, the pump will be set to not infuse any saltwater during the study period. The reason for the pump to be filled but not infuse saltwater is that the pumps will alarm if there are no solution in the infusion and/or cartridge. Furthermore, we need to mimic that we are refilling the cartridge and infusion sets as we do for glucagon in order to keep the blinding for the participants. The "dummy" pump will only serve as a placebo to glucagon and will not follow an algorithm. Therefore, sodium chloride is considered a study tool and not a study medication.

8. Study outcomes

8.1. Phase 1

No specific endpoints. In this phase, we explore the operability of our systems with emphasis on the safety issues.

8.2. Phase 2

8.2.0. Primary outcome between single- and dual-hormone closed-loop system

- Percentage of time with glucose values < 3.9 mmol/l as measured by CGM*

8.2.1. Secondary outcomes between single- and dual-hormone closed-loop system

8.2.1.1 Carbohydrate outcomes

- Number of carbohydrate interventions to treat hypoglycemia*

8.2.1.2. Glucose outcomes

- Percentage of time with glucose values in the range 3.9-10.0 mmol/l measured by CGM and YSI*
- Percentage of time with glucose values < 3.9 mmol/l as measured by YSI*
- Percentage of time with glucose values in the range > 13.9 mmol/l measured by CGM and YSI*
- Percentage of time with glucose values < 3.0 mmol/l as measured by CGM and YSI*
- Mean blood glucose value measured by CGM and YSI*
- Number of hypoglycemic episodes < 3.9 mmol/l on CGM an YSI
- CGM glycemic variability measured as SD and CV*
- Composite outcome: Percentage of participants achieving (1) time in range (3.9-10) > 70 %, (2) time in alert hypoglycemia (<3.9 mmol/l) < 4 %, and (3) time in clinical hypoglycemia (<3.0 mmol) < 1% as measured by CGM and YSI*

8.2.1.3. Insulin and glucagon dosages

- Total insulin dose
- Total glucagon dose
- Number of manual insulin boluses

8.2.1.4. Adverse events

- Number of adverse events (event if visual analog scale (0-100) increase >10 from baseline) for
    - Nausea
    - Headache
    - Palpitation
- Number of vomits

8.2.1.5. Other:

- Difference between actual and participant-estimated CHO content in meals
- Mean Borg scale level during exercise

- Physical activity intensity measured by ActiGraph GT9X Link
- Sleep efficiency measured by ActiGraph GT9X Link

Means, medians, percentages, standard deviations, ranges, interquartile ranges and 95% confidence intervals will be calculated for descriptive analyses. All inferential outcome analyses compare single-hormone with dual-hormone closed-loop glucose control using paired t-test for normally distributed data and Wilcoxon signed-rank test when data cannot be assumed to be normally distributed.

* An asterisk indicates that the outcome will be calculated for the whole study period as well as for the following intervals: dinner (19:00-23:00), night (23:00-08:00), breakfast (08:00-12:00), lunch + snack (12-16:30), and exercise session (16:30-19:00).

9. Statistical considerations and power calculation

9.1. Phase 1

Phase 1 is a feasibility study in which we explore operability of our system with special emphasis on safety issues. Reaching statistical significance is not a goal. We choose to include a maximum of four participants with T1D in this phase.

9.2. Phase 2

To be able to detect a difference in percentage of time with glucose values < 3.9 mmol/l (primary outcome) of 2.3 %-points (approximately 30 minutes) with 90% power, a 5%-significance level, and a presumed 3.0 %-points standard deviation, 20 participants should be included in the study (2-sided test). The sample size calculation is partly based on data obtained from our adult study; however, we expect higher variation due to pubertal status.

Participants who drop out of the study will be replaced such that the total number of participants who have completed a single- and a dual-hormone closed-loop study is 20. Only data from participants who have completed a full single- and a full dual-hormone study session will be included in the analysis comparing the two control types. More than 80% of possible sensor values from each study session are required to be included in the analysis. Furthermore, data from completers and non-completers will be stated in the final report.

For every carbohydrate rescue during the study, 30 minutes of the time in range is allocated to time in hypoglycemia and will be accounted for in the primary analysis. A paired student's t-test will be used to compare the outcomes if data are normally distributed. Otherwise, a Wilcoxon Signed Rank test is be used. In order to account for missing values and adjusting for different factors, a repeated measurement ANOVA will be used with the outcome and adjusting factors as fixed effects and the intercept as a random effect. Bonferroni adjustments will be applied for non-specified outcomes.

Based on previous experience from this type of study we expect a very low drop-out rate, < 10%.

Therefore, a maximum of 24 participants are needed for this project that includes phase 1 (N=4) and phase 2 (N=20).

If it turns out that deviations from the statistical plan described above are necessary, the Danish Health and Medicines Authority, the Regional Scientific Ethics Committee and the GCP-unit will be informed. The original statistical plan as well as the new strategy will be reported in the publication of study results.

10. Safety

For safety reasons, at least one study personnel will always present during the clinical study.

10.1 Hypoglycemia management:

Hypoglycemia will be managed according to the standard operating procedures (SOP_Dysglycemia). A hypoglycemia episode is defined as one plasma glucose value < 3.0 mmol/l. In the event of a hypoglycemic episode, the attending physician will take immediate action to raise the blood glucose by providing the participant with oral carbohydrate. The treatment will be repeated if the hypoglycemia has not resolved within 15 minutes. Repeated YSI measurement will be performed every 5 minutes until BG is > 3.9 mg/dl (SOP_Dysglycemia).

If at any point during the study the participant experiences symptoms of hypoglycemia and requests oral carbohydrate, a blood sample for plasma glucose determination will be drawn. Irrespective of the plasma glucose value the participant will be treated with oral carbohydrate. However, repeated YSI measurements will only be measured if < 3.9 mmol/l.

If at any point during the study the participant experiences a severe hypoglycemic event, i.e. the participant is unable to take oral CHO, intravenous glucose will be administered via the sampling cannula and the study will be terminated.

10.2. Hyperglycemia management:

Hyperglycemia will be managed according to the standard operating procedures. If an YSI blood glucose value exceeds 14.0 mmol/l or if YSI blood glucose values are > 12.0 mmol/l for > 2 hours, blood ketones will be measured using a Precision Xceed ketone meter (Freestyle Precision B-ketone, Abbott, Abbott Park, Illinois). If the ketone level is ≥ 0.6 mmol/l, the closed-loop system will be checked for any malfunction and any problems will be corrected (SOP_Dysglycemia). Ketone measurements will be repeated every 15 minutes until the ketone level is < 0.6 mmol/l. However, if hyperglycemia occurs within 2 hours postprandially, no ketone or other actions must be done.

10.3. Adverse Event Management – Study Medication

Definition of adverse event (AE): Any untoward medical occurrence in a participant or clinical investigation subject administered/using a product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not considered related to the product. Hyperglycaemia and hypoglycaemia 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 (note: the term life-threatening refers to an event in which the participant was at risk of death at the

time of the event; it does not refer to an event which hypothetically might have caused death if it 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 refer to sections 4.8 of the medication summaries for GlucaGen® and FiAsp (attached).

Classification of an AE: Related or not related.

Final outcome classification of an AE: Recovered or not recovered.

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. This includes events occurring during the clinical studies i.e. two times 26 hours, and in the 50 minutes following each study (corresponding to five half-times for glucagon). SAEs, SARs, SUSARs will initially be reported to the sponsor within 24 hours after the investigator has become aware. All follow-up data will be described in detail in a SAE-formula and hereafter the formula will be given to the sponsor within 24 hours of obtaining the new knowledge. Previous non-serious AEs, which becomes SAEs follows the reporting of SAEs.

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.

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.

10.4. Adverse Event Management – Medical Devices

The definition of a serious adverse event is given above ('adverse event management – study medication' section). With regard to medical devices, serious adverse events also include situations that could potentially have led to serious adverse events if circumstances had been different or if there

had been no interference, so called near serious adverse events (NSAEs). However, hypoglycaemia and hyperglycaemia events handled according to our safety procedures are not regarded as NSAEs.

Reporting of SAE and NSAE: All events meeting the definition of a SAE or a NSAE will be collected and reported. This includes events occurring during the clinical studies i.e. two times 26 hours. In case of SAE or a NSAE, the primary investigator should be informed within 24 hours (contact information on page 2). All follow-up data will be described in detail in a SAE/NSAE-formula. The primary investigator will ensure that all information on adverse events that are/could have been 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 event. Within eight days after reporting, sponsor will provide the Danish Health and Medicines Authority with all relevant information about the primary investigator's response to the alert and consequences for the further course of the study conduct.

When reporting SAEs and NSAEs to the authorities the following parameters are included: Study name, participant identification (subject number, initials, sex, age), event (preferably a diagnosis), study medication, reporter identification (name or initials), causality and outcome.

Reporting at the end of the study: All SAEs and NSAEs will be included and be uploaded in the EudraCT. The link 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.

11. Source data

A separate electronic case report form (eCRF, RedCap) will be prepared for each study participant.

Participant information will be retrieved from hospital databases (Sundhedsplatformen, Labka, Carelink, Diasend, Libreview, Medtrum Easyview). For identification and recruitment, we will collect the following information: diagnosis, diabetes duration, age, sex, insulin treatment modality, glucose sensor use, last HbA1c, comorbidities, and allergies. For baseline characteristics and for having information on factors that might impact the performance of the closed-loop system, we will collect information such as the participant diagnosis, age, sex, diabetes duration, treatment modality, insulin pump settings, HbA1c-level, kidney and liver parameters, diabetes complications, and medications.

Venous blood glucose measurements are documented by the apparatus (YSI, Yellow Spring Instruments 2900 STAT Plus) excel files.

Data from participants' own pumps are uploaded to the Medtronic CareLink database, Diasend database or Medtrum Easyview. A unique user profile will be generated for each participant.

Information from the smartphone such as CGM-values, insulin dose and glucagon dose will be stored electronically on the communication platform shown previously (see section on communications platform).

Data from the Actigraph are stored locally and will at the end of the study visit be uploaded to the local research server and transferred to the participant's eCRF.

Data from devices without long-term internal memory, such as the blood pressure apparatus, will be transferred directly to the eCRF. Other data going directly into the eCRF include VAS-scores, self-assessment of glucose values and participant estimates of CHO content.

Any deviation from the protocol will be documented in the eCRF and further be reported to the Danish Medical Agency if required.

A monitoring plan will be established in collaboration with the GCP-unit at Copenhagen University Hospital and includes full monitoring of informed consent, power of attorney, serious adverse events, inclusion and Exclusion criteria. Source data will also be verified by the GCP-unit, according to the monitoring plan. In addition to the informed consent, participants will provide a power of attorney to allow the GCP-unit and the Danish Medicines Agency to inspect and monitor this study.

Data will be stored in coded form in ten years after last participant last visit according to recommendations from the national regulators.

11. Biobank

No biobank will be established. All blood samples will be analyzed immediately after they have been drawn and discarded after analyses.

12. Study rescheduling and termination

A study might be discontinued due to technical challenges, adverse events, an episode of severe hypoglycemia or participant request. Rescheduling is possible if the cause of discontinuation is modifiable according to the investigator's assessment.

The study as a whole may be terminated in the event of unresolvable technical issues such as algorithm underperformance or other unforeseen events that by the investigator's evaluation makes study continuation unjustifiable.

If the technical issues require to change the medical devices, the sponsor will send an amendment to the Danish Medical Agency for a renewed approval before study continuation.

14. Time schedule

First participant first visit is expected in April 2021. Last participant last visit is expected in June 2021. Analysis of blood samples and data will be completed before April 2023.

15. Project economy

The project is investigator initiated. Investigator salaries, equipment, medications and consumables are covered by the research unit at Steno Diabetes Center Copenhagen and Technical University of Denmark. None of the investigators have personal financial interest in the conduct or the outcome of the project.

The primary investigator, Ajenthen Ranjan, is employed in a 2-year PostDoc position at Steno Diabetes Center Copenhagen and funded by the Danish Diabetes Academy Grant ID PD002-19. The study has further received a research grant from the Danish Diabetes Association (225,000 DKK).

Further funding will be applied for. If further funding is obtained, the Regional Ethics Committee will be informed, and the participant information will be updated accordingly.

16. Remuneration

Participants receive DKK 750.- after each study visit completion. Thus, a total of DKK 1500.- will be given if both study visits have been completed. If choosing to withdraw before study end, the participants with the receive the remuneration equal to the number of completed study visits. The remuneration is compensation for discomfort and troubles by drawing blood samples due to each study visits, which are more frequent than during routine clinical care. At the research facility, access to the hospital WIFI and television will be provided, as well as, food, coffee, tea and water. Participants do not gain better treatment during the study period. In addition, travel expenses are covered if individuals living more than 10 km away with a maximum of DKK 500.- per visit. If driving, a rate of DKK 1,90 per kilometre is given.

17. Risk assessment

During closed-loop glucose control, the glucose sensor provides input to the control algorithm and therefore the performance of the system is dependent on reliable glucose sensor values. Although the accuracy of the DexCom G6 CGM is high[41], there may at times be differences between the subcutaneous glucose value measured by the glucose sensor and the actual blood glucose value. If the glucose sensor reads higher than the actual blood glucose value, the control algorithm may calculate an insulin dose that could induce hypoglycemia. The glucose sensor calibration procedures described above are constructed such that hypoglycemia induced by insulin overdosing due to an inaccurate sensor is prevented insofar as possible. If the participant, despite these safety measures, should experience symptoms of hypoglycemia, the attending study personnel will immediately take the necessary steps to avoid a severe hypoglycemic episode (SOP_Dysglycemia).

The algorithm has been extensively tested and in general is very conservative regarding insulin dosing and therefore the risk of inappropriate insulin and/or glucagon dosing is low. Still, a study personnel is always present during the study to overlook glucose values and the condition of the participant and the study personnel may intervene by halting the insulin delivery, give extra glucagon or give intravenous glucose to prevent severe hypoglycaemia.

Due to the small doses of glucagon there is a small risk of glucagon pump infusion tube clotting. Clotting would prevent delivery of appropriate amounts of glucagon which in turn could lead to hypoglycaemia. If the attending study personnel suspects clotting, for instance if the participant does not react to glucagon administration as expected, the glucagon infusion site and tube should be changed.

According to the ICH GCP 4.3.1, the study investigators are responsible for the medical decisions. Thus, investigators can at any time remove the participant blinding of the medication if clinically needed.

Insertion of the sampling cannula may inflict a transitory pain and a risk of a small hematoma. Similarly, the insertion of the pump infusion sets and the CGM may inflict a transitory pain, and a minimal risk of infection at the CGM insertion site exists. Participants are familiar with this potential discomfort from their usual treatment procedures. Once the sampling cannula, infusion sets, and CGM are in place, there should be no significant discomfort.

The net blood loss from screening and the two study sessions is < 120 ml and should cause no symptoms of anemia or hypovolemia.

Glucagon administration may induce nausea and vomiting, however, as dose sizes in this study are small, the frequency of these side effects is expected to be low.

With regard to all other planned study procedures, the risk of complications or adverse events is negligible.

A trained study personnel will always be present at the research facility during conduct of the study. The trained study personnel can always contact the study investigator, who will be with them within short time. Initially, only one participant will be investigated at a time. Afterwards, we may decide to investigate two participants at a time. One medically trained study personnel will be present per study participant.

18. Participant insurance

Participants are covered by the mandatory participant insurance, "Patienterstatningen", at Steno Diabetes Center Copenhagen. This means that participants have the right to complain about the treatment given and that they are entitled to compensation in case of malpractice.

19. Ethical considerations

The expected short-term benefits of dual-hormone closed-loop control are improved glucose control with less hypoglycemia and more time in the target range as well as physiological and psychological well-being in participants with T1D due to less glycemic variation and reduction of cognitive load.

The study will be carried out in accordance with the Helsinki Declaration and the principles of good clinical practice after approval by the Regional Scientific Ethics Committee, and the Danish Health and Medicines Authority. 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 participant journals, during monitoring, auditing and/or inspection by an ethics committee, the Danish Health and Medicines Authority or any other countries' health authorities, respectively.

Data are managed in accordance to the Regulation (EU) 2016/679 of the European parliament and of the council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC (General Data Protection Regulation). Procedures for quality assurance and quality control will be applied according to local guidelines.

20. Precautions on privacy and physical and mental integrity of study subjects

All information on study subjects is protected according to law on processing of personal data and the law of health. The electronic study database is password protected and located on the hospital network server which is continuously backed up. CPR-numbers are substituted by numeric codes in the database. The key linking the CPR-number and all other information that is personally identifiable with the numeric code will be kept in a locked filing cabinet in the sponsor's locked office. Only the study sponsor and investigators will have access to the study database. 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.

21. Dissemination of study results

Study results, positive, negative and inconclusive findings, will be presented at national and international scientific meetings and published in international scientific journals. Ajenthen Ranjan will be first author and Kirsten Nørgaard as last author on all publications arising from the clinical part of this study. On the other hand, Asbjørn Thode Reenberg will range as first author and John Bagterp Jørgensen as last author on all publications arising from the technical part of this study. Inclusion of other authors will depend on individual contributions in keeping with the Vancouver Protocol.

In addition, summary results will be communicated to study participants by letter and to the T1D population in general via the Danish Diabetes Association.

After conclusion of the study, a final report will be generated and sent to the Regional Scientific Ethics Committee and the Danish Health and Medicines Authority.

22. References

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23. Appendix

23.1. Worksheet – Standard Operating Procedure

23.2. Dysglycemia – Standard Operating Procedure

23.3. DiaCon algorithm – Standard Operating Procedure

23.4. DiaCon Closed-loop system – Investigator's Brochure

23.5. Dana Diabecare RS® insulin pump – Instruction for use

23.6. DexCom G6® CGM – Instruction for use

23.7. DiaCon Closed-loop system – Instruction for use

23.8. Glucagon® – Medication summary

23.9. FiAsp® – Medication summary