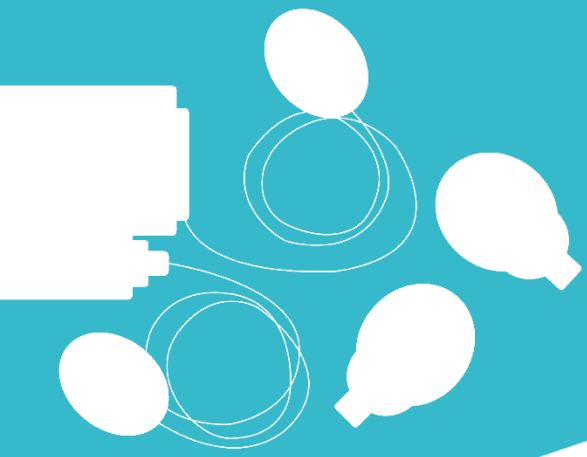
Advancing Bihormonal Closed-Loop Systems: The Role of Glucagon in Glucose Management of Diabetes Mellitus Type 1

Master thesis



L.J.J. van den Berg

Technical Medicine - Medical Sensing & Stimulation - University of Twente
Internal Medicine, Rijnstate Hospital Arnhem
Inreda Diabetic, Goor

May 2023 - March 2024

Supervisors

dr.ir. B.J.F. van Beijnum	Chairman and Technical supervisor, University of Twente
dr. A.C. van Bon	Medical supervisor, Rijnstate hospital Arnhem
L.B. Vlaskamp, MSc	Daily supervisor, Inreda Diabetic
drs. N.S. Cramer Bornemann	Process supervisor, University of Twente
dr. J.K. van Zandwijk	External member, University of Twente

Inreda®

UNIVERSITY OF TWENTE.

Rijnstate

Preface

Beste lezer,

Het afgelopen jaar heb ik met veel toewijding gewerkt aan mijn masterthesis op de afdelingen interne geneeskunde en vasculair centrum van het Rijnstate Ziekenhuis te Arnhem en Inreda Diabetic te Goor. Ik ben verheugd om het resultaat met u te kunnen delen. In de periode van mei 2023 tot april 2024 heb ik met veel enthousiasme en uitdagingen mijzelf ondergedompeld in de wereld van diabetes, data verwerking en professionele groei. Ik ben dankbaar voor de hulp die ik van iedereen heb gekregen.

In het bijzonder mijn begeleiders: Bert-Jan, hartelijk dank voor uw enthousiasme en betrokkenheid dat u vanaf het allereerste moment hebt getoond. Uw waardevolle adviezen reikten verder dan alleen de technologische aspecten en hebben mij geholpen mijn werk op verschillende gebieden te verfijnen. Lyan, bedankt voor jouw begeleiding en aandacht gedurende dit jaar. Je hebt me niet alleen meegenomen in het werk van een technisch geneeskundige binnen een bedrijf, maar ook de ruimte gegeven om zelf mij weg te zoeken in het onderzoek. Jouw capaciteit om mij op de juiste momenten te laten leren, adviezen te geven en te remmen was van onschatbare waarde voor mijn ontwikkeling. Arianne en het diabetes team, ik wil graag mijn oprechte dank uitspreken voor jullie tijd en aandacht gedurende mijn leerproces in de behandeling van diabetes in de praktijk. In het speciaal Arianne, voor de sturing die je hebt gegeven aan mijn klinische ontwikkeling en het vertrouwen wat jij mij daarin heb gegeven. Jullie persoonlijke aanpak richting de patiënten is echt bewonderenswaardig. Ik ben dankbaar dat ik voor één jaar een klein onderdeel van jullie team mocht zijn. Nicole, bedankt voor de afgelopen twee jaar waarin je mij aanzette om anders naar situaties te kijken, mij hielp te groeien als professional en als persoon, en mij aanmoedigde om regelmatig terug te kijken.

Daarnaast wil ik Inreda Diabetic en het Rijnstate bedanken voor de kans die zij mij hebben geboden om mijn afstudeerstage te voltooien. Helga Blauw bedanken voor de begeleiding in mijn eerste maanden en Jordy van Zandwijk voor het vervullen van rol als buitenlid van de afstudeercommissie. Ook wil ik de collega's bedanken voor hun betrokkenheid en gezelligheid gedurende deze periode.

Ik hoop dat deze thesis een waardevolle bijdrage zal leveren aan een verbeterde behandeling en verdere ontwikkeling van de Inreda AP. Hierdoor kunnen in de toekomst nog meer patiënten geholpen worden en kan de ziektelast als gevolg van diabetes verder verminderd worden.

Loes van den Berg

Maart 2024

Abstract

Introduction
In the past decades, there has been a growing interest in incorporating glucagon into continuous subcutaneous insulin infusion therapy for Type 1 Diabetes Mellitus patients. However, widespread adoption as a standard treatment option has not yet been realized. The development of bihormonal closed-loop systems involves investigating the pharmacodynamics of glucagon in controlled settings. While some studies have examined these systems in real-life settings, the focus has primarily been on their overall success in regulating glucose levels within target ranges. Limited information is available on the real-life impact of glucagon on glucose levels.

Objectives
This study aims to assess the effect of glucagon on glucose levels in real-life data, exploring various conditions and investigating the impact of glucagon settings in the Inreda AP, the first CE-certified bihormonal closed-loop system. The objective is to gain deeper insights into the role of glucagon in glucose management, comprehend glucagon aspects within treatments with the Inreda AP, and explore potential optimizations for enhanced effectiveness.

Methods
Glucose responses to glucagon are systematically mapped under various conditions, including control (blockage), insulin on board, and different situations (baseline glucose value, rate of glucose change and amount of glucagon) and patient characteristics (gender, age, BMI, diabetes duration, HbA1c, total daily insulin dosages and insulin sensitivity). The impact of alterations in system settings on regulatory outcomes is also examined. Cluster analysis is applied to predict glucagon settings.

Conclusions
This research demonstrates that, in real-life scenarios, glucagon administration can lead to earlier and more pronounced increases in glucose levels. The presence of insulin on board mitigates glucose excursions after glucagon dosage, yet the pre-dosage rate of glucose change appears to be a more decisive factor. Moreover, patients with lower insulin requirements exhibit greater glucose excursions compared to those with higher insulin needs. Although there is no one-size-fits-all glucagon setting, reducing settings results in decreased individual glucagon usage. Practical recommendations for treatment adjustments and algorithm refinements are derived from this study, offering valuable insights to enhance the effective use of glucagon in glucose regulation.

CONTENTS

1 Introduction 6

2 Background 8

3 Effect of glucagon dosages on glucose levels in uncontrolled settings 13

4 Interaction of Glucagon and Insulin on glycemic control 21

5 Intra- and inter-variability in glucose response of glucagon 28

6 Glucagon release in the bihormonal closed-loop system 36

7 Patient Clustering for Optimized Glucagon Settings 45

8 Advancing the use of glucagon in bihormonal closed-loop system 49

9 General discussion 52

References 56

Appendix A 60

Appendix B 62

Appendix C 66

Appendix D 68

Appendix E 70

List of Abbreviations

AP	Artificial Pancreas
AUC_{80min}	Area Under the Curve in 80 minutes frame
AUC_{delta}	Area Under the Curve till baseline glucose is restored
BGM_0	Baseline Glucose Measurement
BMI	Body Mass Index
CGM	Continuous Glucose Monitoring
Curve	curve setting or injections based on curve criteria
EH	units
EI	Extra injection
G_{max}	Maximal glucose excursion
G_{min}	Minimal glucose excursion
HbA1c	Glycated hemoglobin
Inreda AP	Bihormonal closed-loop system of Inreda Diabetic
IQR	Interquartile Range
N	Number of patients
#	Number of clusters
PID	Proportional–integral–derivative
Preceding Slope	Rate of change of glucose levels before glucagon injection
$Slope_{10min}$	Rate of change of glucose levels in 10 minutes before first glucagon injection
T1DM	Type 1 Diabetes Mellitus
TAR	Time Above Range
TBR	Time below Range
TDD	Total Daily Dose
TDD_g	Total Daily Glucagon Dose
TDD_i	Total Daily Insulin Dose
$TDDi_{AP}$	Total Daily Insulin dose during AP treatment
$TDDi_{bAP}$	Total Daily Insulin dose before AP treatment
T_{delta}	Time till baseline glucose is restored
TIR	Time In Range
T_{max}	Time till maximal glucose excursion
T_{min}	Time till minimal glucose excursion
U_g	Amount of glucagon dosed in one cluster
VI	Correction injection

1 Introduction

Every day, ten patients are diagnosed with diabetes mellitus type 1 (T1DM) in the Netherlands [9]. Currently, the Netherlands counts around 100.000 patients with T1DM. The numbers of patients are increasing not only in Western countries but worldwide [10, 14]. Patients are assigned to managing their own blood glucose every single day for their whole life [7]. Moreover, badly regulated diabetes comes with greater risk of complications. The life expectancy of patients with diabetes is significantly reduced, 13 years on average for a 45 years old person [26]. T1DM has a major impact on the society but even more on the quality of life of the patients [14, 21].

Normally, the blood glucose level is automatically regulated by the body, while this is dysregulated in patients with T1DM [19]. The auto-immune disease deteriorates the Beta-cells of the pancreatic islets which are responsible for the release of insulin. The hormone insulin ensures that available glucose by intake of carbohydrate will be taken up into the cells of the target organs and therefore reduces the blood glucose levels. T1DM should be treated with insulin to control the blood glucose levels. High blood glucose levels affect the macro and micro vascularity especially those of the nerves, retina and kidneys, which may lead to neuropathy, blindness and kidney failure. The better the blood glucose is regulated in a normal range, the less risk of complications in the long term [29]. However, the blood glucose levels can also be too low, also known as hypoglycemia [8]. When this happens, there is not enough energy to serve all tissues and people can feel shaky, nauseous, fatigue and anxious. If the hypoglycemia worsens patients can get confused and this could even result in loss of consciousness and seizures. This can be fatal if not treated immediately, but preferably to prevent these situations in time.

The blood glucose in patients with T1DM is controlled by use of exogenous insulin [17]. Four decades ago, the patient had to do multiple finger prick tests per day to measure blood glucose level and inject themselves with insulin using a syringe or later a pen [24, 17]. The patient has to take into account multiple factors, like activity and amount of carbohydrates consumed, to calculate the dosages [34]. In the late seventies, the first wearable insulin pump was designed wherefore it was possible to induce insulin in a more gradual manner [17]. It took a couple of decades to improve it to their current design and performance. Developments in glucose measurements came with the flash glucose monitoring, patients only have to scan the sensor with their phone or reader to obtain glucose values. Continuous Glucose Monitors (CGM) have transformed diabetes care by providing patients with real-time glucose levels without the need for manual scanning. This eliminates the gaps between finger pricks, allowing users to effortlessly monitor their glucose levels continuously [24]. Further steps are undertaken in communication between the sensor and the pomp. The latest developments are those of the hybrid closed-loop and the fully automatic closed-loop systems, whereby the patients should announce meals or no longer in case of the fully closed-loop systems. All these devices are nowadays still used depending on indication of the devices and preference of patients.

Nonetheless the technology has made substantial improvements for care of T1DM, the improvements are only focused on insulin. This limits the ability to achieve more time in normal blood glucose levels, time in range (TIR), without increasing the risk of hypoglycemia and to regulate patients with strong fluctuating blood glucose levels [25]. It takes some time before the insulin starts to act and thus lowers the glucose level. The effect of insulin continues for 5 till 8 hours depending on kind and dosage [11]. This can result in unintended hypoglycemia and patients must consume carbohydrates at that moment to raises the blood glucose levels. Therefore, insulin is dosed with caution to prevent hypoglycemia which contributes to higher average blood

glucose values.

A solution to increase the blood glucose levels is to use glucagon, the counteracting hormone of insulin [25]. In a healthy person's pancreas, glucagon is produced by the alpha cells and stimulates hepatic glucose production by increasing glycolysis and gluconeogenesis [16]. Inreda Diabetic has developed the first CE-certified artificial pancreas (Inreda AP) with bihormonal closed-loop regulation without the need for meal announcements [3]. Blauw already studied the effect of glucagon for several doses at different blood glucose levels in a controlled setting [4]. This study suggests that glucagon has a dose-dependent effect on glucose excursions. Very little is currently known about the response to glucagon in uncontrolled settings [25]. This limits the ability to understand and therefore optimize (individual) treatment with the Inreda AP. Healthcare professionals and advisors of Inreda are making decisions based on the knowledge and experience with insulin and logical reasoning. However, this leads to a trial and error approach, reduced ease of use and discrepancy between professionals.

The Inreda AP is already applied in larger studies performed in outpatient settings and besides studies used as regular care in 125 patients for two years. For more than 7000 days of glucose, insulin, glucagon, alarm and accelerometer data is available. Hence, a lot of data is available to analyze the effect of glucagon dosages in uncontrolled settings. This thesis will examine the emerging role of glucagon in the context of the Inreda AP. Comprehending glucagon in uncontrolled setting could help to optimize glucagon settings in the individual, support the ease of use and possibly improve the algorithm. A personalized approach might be implemented for glucagon settings which results in better glycemic control and less burden on the patient. Moreover, it could provide more guidelines for healthcare providers to start or adjust treatment and for developers to optimize the control algorithm.

This thesis will first discuss a brief background about the pathophysiology of T1DM and the AP control system. Thereafter, the performed studies are discussed. In **Chapter 3**, the effect of glucagon on the glucose level will be examined. In **Chapter 4**, the influence of insulin on board will be taken into account in reviewing the effect of glucagon on glucose levels. **Chapter 5** examines glucose excursions by multiple inter- and intra-patient variables to gain insight into crucial factors influencing these excursions. **Chapter 6** will focus on glucagon settings of the Inreda AP and how this affects the treatment. In **Chapter 7**, a clustering method is applied and reviewed to predict glucagon settings in individual patients in order to improve personalized diabetes management. **Chapter 8** is concerned with some suggestions to advance the use of glucagon in the Inreda AP.

1.1 Objectives

The objectives of the study are stated as follows:

1. Assess and quantify the impact of glucagon administration on glucose levels.
2. Analyze the interaction between insulin on board and the effectiveness of glucagon.
3. Evaluate potential factors, both within and between individuals, that play a significant role in influencing the response to glucagon.
4. Investigate what impact glucagon settings have on the glucose regulation.
5. Apply and evaluate a cluster method for predicting optimal glucagon settings.
6. Propose guidelines to optimize the utilization of glucagon within the Inreda AP.

2 Background

This chapter provides more details about the pathophysiology of diabetes Type 1 and the control mechanism of the AP.

2.1 Pathophysiology of diabetes Type 1

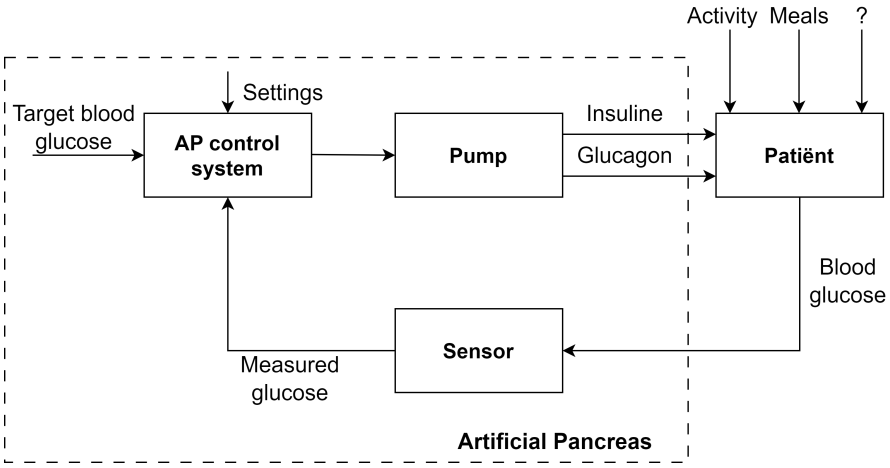
T1DM is an organ-specific autoimmune disease where insulin-producing beta cells in the pancreas are attacked by their own body. As a result, there is no production of the hormone insulin and this disturbed the carbohydrate metabolism. Insulin takes care of the uptake of glucose from the blood into the cells and the storage of glycogen, the stored form of glucose. After ingestion of food, the excess of glucose in blood can be stored or converted into energy which ensures a stable blood glucose level. These processes cannot take or take insufficient place and therefore a state of hyperglycaemia, a blood glucose level >10 mmol/L, can be reached. Patients with DM1 should be treated with exogenous insulin to reduce the blood glucose. Managing insulin dosing is challenging because of the complex interplay of factors affecting insulin sensitivity and estimation of carbohydrates and meal composition. An excess of insulin can decrease the blood glucose till a life treating low level. Patients should eat carbohydrates to raise their glucose levels. In healthy humans, the glucose metabolism has an counteracting hormone which prevent hypoglycemia from happening. Glucagon is produced in the alfa cells of the islets of Langerhans in the pancreas. Glucagon is released in the portal vein which go straight to the liver where it will stimulate glycogenolysis and gluconeogenesis. Both transforming glycogeen and other resources to glucose which increase the blood glucose.

There are several theories why the counteracting mechanism is absent or is not able to sufficient rises the blood glucose in T1DM [31]. 1. Paracrine signaling is lost due to beta cell loss. Paracrine signaling is a form of cell communication in this case the neighboring beta cells. 2. Intrinsic glucose sensing abilities are redundant in type 1 diabetes. This theory has the idea that the cells are insensitive to changes in glucose levels and therefore also glucagon is not produced. 3. Excessive Delta somatostatin secretion lead to suppression of alpha cells. The pancreas consist about 10% of Delta cells which secrete somatostatin, which should suppresses the activity of alpha cells. 4. Islet autonomic innervation of glucagon secretion. This is suspect to not be critical for glucagon secretion. 5. Another hypotheses is a state of hyperglucagonemia due to hypersecretion of glucagon by loss of inhibitory beta-cell control [5]. This theory acknowledge a more glucagonocentric approach as key hormone in T1DM then the conventional insulineocentric view. Most of the theories are not proved or only has been supported in animal studies [31].

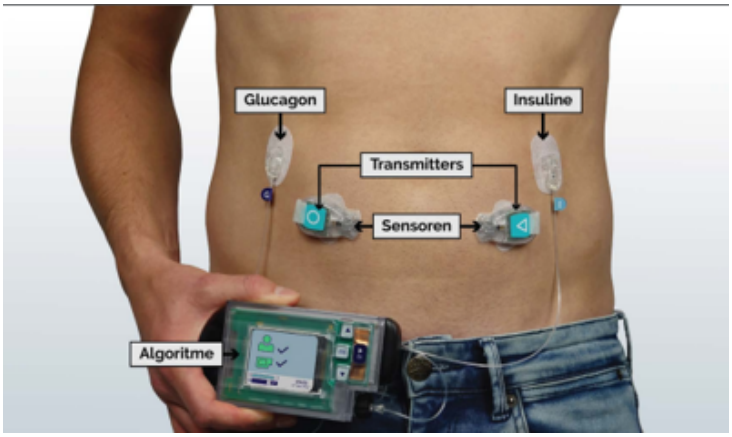
2.2 Bihormonal closed-loop algorithm

The bihormonal closed-loop system is a dynamic system, as the same goes for the glucose metabolism in the human body. Figure 2.1a shows a schematic overview of a closed-loop control system with the AP and Figure 2.1b shows the realistic representation. Glucose levels of a patient can fluctuate throughout the day due to various factors such as meal intake, physical activity and administration of insulin and glucagon. These glucose levels are measured with the glucose sensor, which will be used as input for the AP control system. The transmitter on top of the sensor will filter the raw signal and sent it to the AP control system. The measured

glucose will be compared to a target glucose level. If the target glucose level is not met, then the AP algorithm would determine which hormone and would calculate what amount should be administered. The system can be personalized with parameters (insulin and glucagon settings) which impact the amount of hormone which will be released. The control algorithm sent a request to the motor of the pump to deliver insulin or glucagon into the patient. The hormone will be administered through the infusion line into the patient. The hormone administered ensures that the body initiate physiological processes to increase or decrease the glucose levels. Subsequently this change will be measured, and the new glucose level is utilized as new input for the AP control system. The components of this dynamic system are discussed in detail below:



(a) Schematic scheme control system



(b) Inreda AP components

Figure 2.1: Bihormonal closed-loop system

2.2.1 Sensor

The Inreda AP requires two sensors to increase the reliability of the glucose levels. The sensors have a warm-up time of 2 hours, after that time the sensors can be used as input for the algorithm. Before transferring the data to the AP, the sensor's transmitter filters the measured values with a filter to minimize the impact of noise. Three parameters are extracted from the sensor signal: the absolute glucose values, slope of glucose levels and a measure of reliability. The absolute values are measured every 10 seconds. The slope refers to the rate of change of glucose levels and are calculated over the past XXXX minutes. A measure of reliability is calculated for both sensors and the sensor with the highest reliability is chosen as primary sensor.

The primary sensor is used as input for the control algorithm.

It is important that the glucose values are reliable as this is the input for the control algorithm to decide which hormone and what amount will be administered. Errors caused by the sensor signal could cause undesired dosages and therefore undesired impact on the glucose levels. To reduce these unwanted dosages, a couple of several strategies have been implemented, as mentioned earlier: two sensors and filtering through the transmitter. In addition, control finger pricks are required after the warm-up period and when glucose values of both sensors deviates substantially. Patients are required to input the glucose value obtained from the finger prick into the AP device. The sensor is calibrated using this finger prick data, unless a substantial error is observed, in which the patient is instructed by the AP to replace the sensor. Moreover, it is not advised to change sensors when glucose values are rapidly increasing or decreasing as the deviation between blood glucose values and subcutaneous values will be significant due to the 5 till 10 minutes delay between blood and subcutaneous glucose concentrations. Furthermore, it is crucial for the transmitters and the AP device to be connected to facilitate the transfer of signals as input into the control algorithm. Despite all measures taken for accurate glucose readings, errors can still occur in the signal due to factors such as persistent noise in the signal or sudden jumps in glucose values caused by a change in primary sensor. To mitigate the impact of the remaining errors and missing readings on the data-analyses in this thesis, the 10-second interval is transitioned to an average over a certain timeframe in most cases one-minute average.

2.2.2 AP control system

The AP control system compared the measured glucose at a certain time with a set point or in this case a target glucose level. If the target glucose level is not met, then the AP algorithm would determine which hormone and calculate what amount should be injected. The control system of the AP uses features of the continuous glucose measurement as absolute glucose level and glucose slope (rate of change). This kind of closed-loop system are called Proportional-Integral-Derivative (PID) [33, 12]. The set of rules are composed by the founder of the Inreda AP and the company has developed this in the last decade.

The dosages of insulin and glucagon are categorized in ”curve” injection, correction injection (VI, originated from the Dutch ”Voor Injectie”) and for glucagon only an extra injection (EI). Curve injections are given at certain glucose values and the calculation of dosage amount based on the glucose value and the curve setting (%). A bigger curve setting will give more insulin or glucagon by equal glucose values. VIs are administered by fast decreasing or increasing glucose values and EIs are administered when curve and VI did not achieve the desired effect on the glucose level. Personal settings for VI and EI are given as maximal amount of units which can be given with VI or EI. When patients start with the AP treatment, insulin settings are determined by the total daily dosage of insulin (TDDi) of the previous treatment. Glucagon settings are always started at the default settings. Settings are optimized in a coaching period and later on by the treatment team or with the self-learning algorithm for insulin settings.

It is important that the control system maintains stability and avoids self-disruption or unintended self-regulation. An example of unintended self-regulation is the jojo-effect: an injection of a hormone may trigger a significant glucose excursion, prompting the administration of the counteracting hormone. This process may be sustained for a longer period of time. If this phenomenon is caused by glucagon, glucagon settings should be manually adjusted to prevent unnecessary dosages of both hormones and increased glucose variability.

Thus, the AP control system compares glucose values with the measured value and calculates, if necessary, what amount of insulin or glucagon should be given. The AP could be personalized with insulin settings for curve and VI, and glucagon settings for curve, VI and EI.

2.2.3 Pump

The pump receives a request to administer insulin or glucagon and try to execute the request. The hormone stored in a cartridge are pushed by a motor through the infusion tube and cannula to deliver the hormone in the subcutaneous tissue. The pump can detect blockages and warns the patient that the insulin or glucagon administration was unsuccessful. The control system is paused until the patient takes action. Anti-blockage dosages are administered to prevent clogging of infusion lines. These are small doses of hormone, insulin or glucagon, administered when no hormone is administered in a certain timeframe but does minimally affect the glucose levels. In addition to blockages, leakages can also occur, typically observed with the insulin infusion set, resulting in the hormone not reaching the subcutaneous tissue. In the case of insulin, this can lead to elevated glucose levels, prompting patients to receive an alarm to inspect the infusion set on leakages.

2.2.4 Patient

Glucagon and insulin injection are regulated from outside of the body, the human body activate several processes depending on what hormone is injected, to adapt the blood glucose level in desired range (3.9-10.0 mmol/L). The effect of these hormones is depending on more than 40 factors like the amount of hormone administered, the amount which is uptaken into the blood-stream but also factors affecting the insulin sensitivity like exercise, stress and time of the day [34].

The reason for the increase in blood glucose levels, and thus the need for insulin, is the consumption of carbohydrates. The AP does not require or rather the patient cannot announce meals in the AP which is still needed in hybrid closed-loop systems. The AP is a reactive system; hence, insulin dosages are determined by glucose signals rather than the quantity and composition of the meal. Glucagon aimed to slow down the decrease of glucose levels to avoid hypoglycemia. Sometimes, hypoglycemia is unavoidable, and the patient receives an alarm when glucose levels drop below 3.5 mmol/L, prompting them to consume rescue carbohydrates to raise glucose levels to the desired range. The data registered by the AP does not include when and what the patients has eaten, which limits the context of the data.

Patients without closed-loop systems are used to manage their blood glucose levels constantly, which demands a lot from these patients. While a closed-loop system eliminates the need for carbohydrate input or anticipation of exercise, patients are still responsible for managing alarms, timely replacement of infusion sets and sensors, and ensuring the correct preparation of glucagon.

In summary, patients cannot interfere with the dosing of insulin and glucagon but actions of the patients as meal intake, exercise and not managing alarms influence the glucose levels.

2.2.5 Role in Addressing Research Questions

This closed-loop system is complex to pinpoint the effect of a single factor due to multitude of unknown factors influencing the glucose levels. Despite this challenge, it is imperative to employ the bihormonal closed-loop system for evaluating the glucagon response in real-life scenarios.

Overcoming the challenge of a closed-loop system, involves effectively utilizing the properties of the Inreda AP. This is implemented as follows to achieve the objectives. Firstly, compare the overall effect of glucagon on glucose levels to similar situations where glucagon should be administered but is not. Therefore, open loop situations created by glucagon blockage are used as control. Secondly, to assess the impact of insulin on board, situations with and without insulin dosage prior to glucagon dosage are compared. Thirdly, to assess potential factors influencing the effect size of glucagon, univariate analysis compares responses between groups with similar characteristics. Fourthly, the impact of glucagon settings are compared between different settings between and within patients at time in ranges, hormone usage and occurrence of glucagon dosage. Lastly, the knowledge from the previous objectives is used to address the last two objectives.

3 Effect of glucagon dosages on glucose levels in uncontrolled settings

3.1 Objective

This chapter will explore the impact of glucagon dosages on the glucose levels in uncontrolled situations, utilizing data from normal daily use of the bihormonal closed-loop system. This data may yield valuable insights of the potential of glucagon in diabetes management, specifically in relation to its immediate impact on glucose levels in real life practice.

3.2 Methods

3.2.1 Dataset

This is a retrospective study which utilized a database of AP users. The database consisted of continues glucose measurements of two sensors, timing and amount of glucagon and insulin dosages and occurrence of infuse blockages. The AP system already had filtered the sensor data, determined the primary sensor and calculated the slope of the glucose curve. The glucose values were measured six times each minute. This study utilized a quantitative methodology, comparing glucose excursions following both glucagon dosages and glucagon blockages. Data of patients with Type 1 diabetes who had used the AP between 31 August 2022 and 31 August 2023, were included.

Users of the AP systems were adults aged between 18-75 year, had a BMI < 35 kg/m^2 and HbA1c < 97 mmol/mol. They had been insulin dependent for at least 6 months before start of treatment. Moreover, there were no existing or suspicions of allergies to insulin Lispro, GlucaGen or known problems with enzym-based glucose sensors. For treatment with the AP, the patients should have stayed in contact with their care team, refrained from misuse of alcohol and drugs and did not need dialysis.

3.2.2 Cluster defining method

To evaluate the glucose response to glucagon dosages, the glucose levels should be reviewed within a specific timeframe following the glucagon dosage. In a closed-loop system, multiple glucagon dosages can be administered in a small period of time as the control system will administer glucagon until the glucose levels return to the desired range. To avoid an excessive inclusion of the effect of these successive dosages, these dosages were grouped into clusters. Instead of analyzing the timeframe after each individual dosage, the timeframe after start of each cluster is reviewed. A cluster was defined as successive dosages given to mitigate the decreasing glucose curve. The method used to cluster these successive dosages, hereafter referred to as the cluster defining method, involved time, slope, and absolute glucose threshold values which were based on the AP algorithm. The threshold criteria were varied and visually inspected for a couple of days to determine the criteria that most accurately assigned the clusters. A new cluster was started when for at least 30 seconds a slope of XXXX mmol/L/h or more, or glucose levels greater than XXXX mmol/l were measured since the previous glucagon dosage. A new cluster was also started when more than XXX minutes has been passed since the last glucagon dosage.

Table 3.1: Cluster algorithm performances

	Sample 1	Sample 2	Sample 3	p-value*
Number of clusters	135	152	147	0.137**
% of days correct	60	67	60	0.963***
% of correct clusters	81	92	80	0.548***
% of missed clusters	6	5	5	0.459***
% of overcounted clusters	13	3	16	0.043***
% sensor error	65	43	66	0.888***

* p-value between sample 1 and sample 2
** Mann-Whitney-U-test
*** Chi-Square test

The cluster algorithm was validated by creating two random and exclusive sample of 30 days of glucose data. The cluster defining method was applied to the data and the clusters were displayed in the daily glucose and dosages plots. The control was done by visual evaluation of the clusters; the author noted the correct amount of clusters, too few counted clusters and too many counted clusters per day. An example of visual evaluation is shown in Appendix A.1 . The samples were statistically compared to ensure that the observed results were consistent with the whole dataset. Table 3.1 presents the performance outcomes of the cluster algorithm. The results show no significant difference between the first and second samples in all outcomes, except for the percentage of assigned clusters that do not represent true clusters. Therefore, a third sample of 30 days is assessed. The percentage of the overcounted clusters of the second sample deviate from the other two samples. Overall, 95% of all true clusters are assigned as cluster. 90% of the assigned clusters are true clusters. 60% of the falsely assigned clusters are caused by erratic sensor readings. Another common reason for incorrectly assigned clusters occur when the clustering criteria have already been met, but an additional dose of glucagon is administered at the end of the cluster.

3.2.3 Data analysis

After determining the clusters, a time window of 10 minutes before and 80 minutes after the first dosage was selected for each cluster. The minutes before were included to observe the rate at which glucose was dropping and the 80 minutes after have been selected based on literature, which suggests that this is the maximum time for the glucose excursion to be at its peak [4, 23]. Clusters were excluded when there was insufficient sensor data as the remaining glucose levels were unreliable to interpret. Threshold values are determined based on the visual examination of several clusters with different percentage missing data while ensuring sufficient number of clusters remain for analysis. Clusters were excluded as data was missing for more than ten consecutive minutes, or when less than 70% of the total time frame was available. Thereafter, the baseline glucose value was calculated by the mean glucose level in a two-minute window, one minute before and one minute after the start of the cluster, in order to reduce the impact of sensor errors. Subsequently, the delta glucose is calculated by subtracting the baseline glucose levels from glucose levels and afterward the delta glucose values were averaged per minute per cluster. By examining the delta glucose, it became possible to compare clusters. The clusters were merged and for each minute, the median along with the first and third quartiles was computed. As controls, clusters were selected in which a blockage was detected by the AP system within 5 minutes from the first glucagon dosage, as in this timeframe blockages could be detected. If blockages were detected later on in the cluster, it was assumed that glucagon was already dosed and an effect of glucagon was noticeable and therefore excluded from the

control group. Control clusters were also merged and median as interquartile range calculated for each minute.

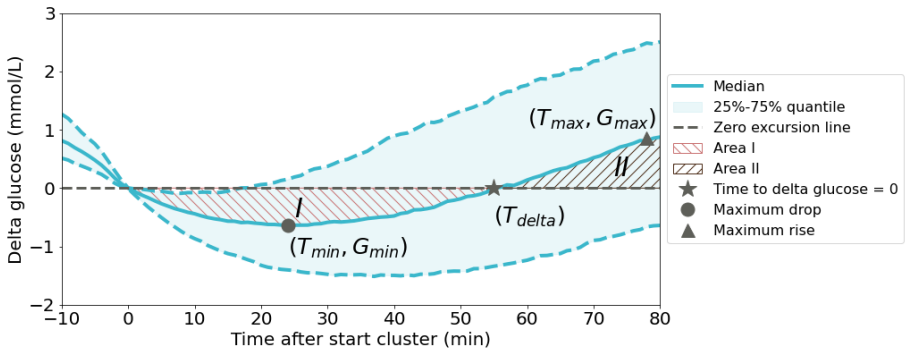


Figure 3.1: Endpoints

The endpoints based on the median delta glucose included in this analysis are illustrated in Figure 3.1 and are labeled as follows: the maximum glucose-drop (G_{min}), time to reach the maximum glucose-drop (T_{min}), the maximum glucose excursion (G_{max}) and time to reach maximum glucose excursion (T_{max}), time till baseline glucose was recovered ($T_{delta\,=\,0}$), total area of 80 minute curve ($AUC_{80\ min}$), consisting of area I and II, see Figure 3.1, the area under the curve till $T_{delta\,=\,0}$, area I in figure, (AUC_{delta}). Moreover, the median and interquartile ranges (IQR) of every 10 minutes were computed. Besides these endpoints, the following information of the glucagon clusters and control clusters were noted: the number of cluster in each group (N), the measured slope of the past 10 minutes ($Slope_{10\ min}$) and the baseline glucose level (BGM_0). Subanalysis were also conducted exclusively on nighttime data, focusing on clusters started between 00:00 and 06:00 hours. Night time cluster analysis were done to reduce the influences of glucose affecting meals and exercise. These endpoints serve as the foundation for our analysis and findings throughout the first three chapters.

3.2.4 Statistical analysis

Non-parametric tests were carried out to compare the distributions of delta glucose values every 10 minutes between the glucagon and control clusters. A p-value <0.05 was considered significantly different. Bonferroni correction was applied for multiple testing. The other endpoints have not been subjected to statistical testing because extracting these endpoints from a single clusters were substantially affected by the erratic sensor patterns. So, meaningful distributions of each endpoint of every group could not been made. Therefore, difference between the groups of the endpoints based on the median glucose response has been assessed for clinical relevance. Data analysis and statistical analysis were performed in Python version 3.11.4.

3.3 Results

3.3.1 Dataset

From the database, 43.311 treatment days from 129 T1DM patients were included in the analysis: 65 male and 63 female, aged between 20 and 72 years at start of the data inclusion. The total number of clusters detected was 231.810 of which 7.708 in the control group and 198.689 the glucagon group, as shown in Figure 3.2. Ten percent of the detected clusters were excluded due to insufficient sensor data.

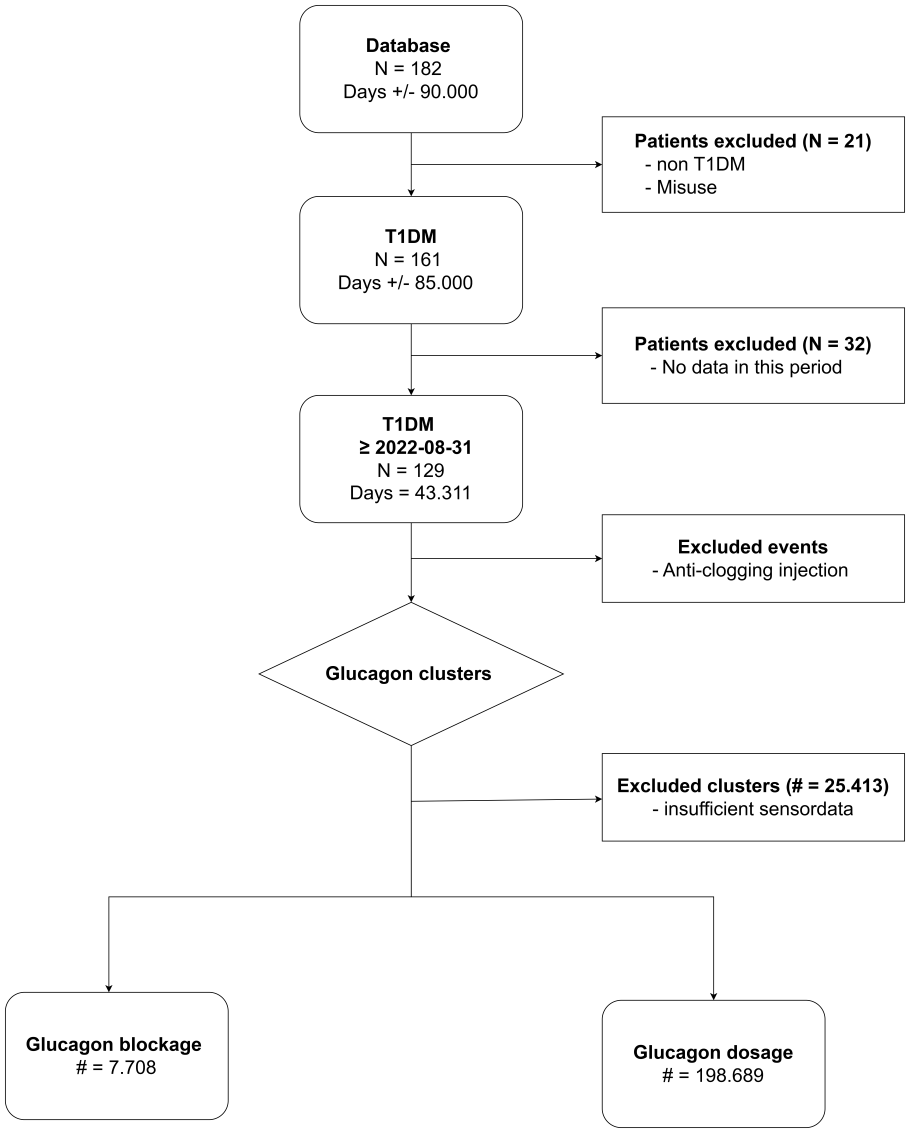
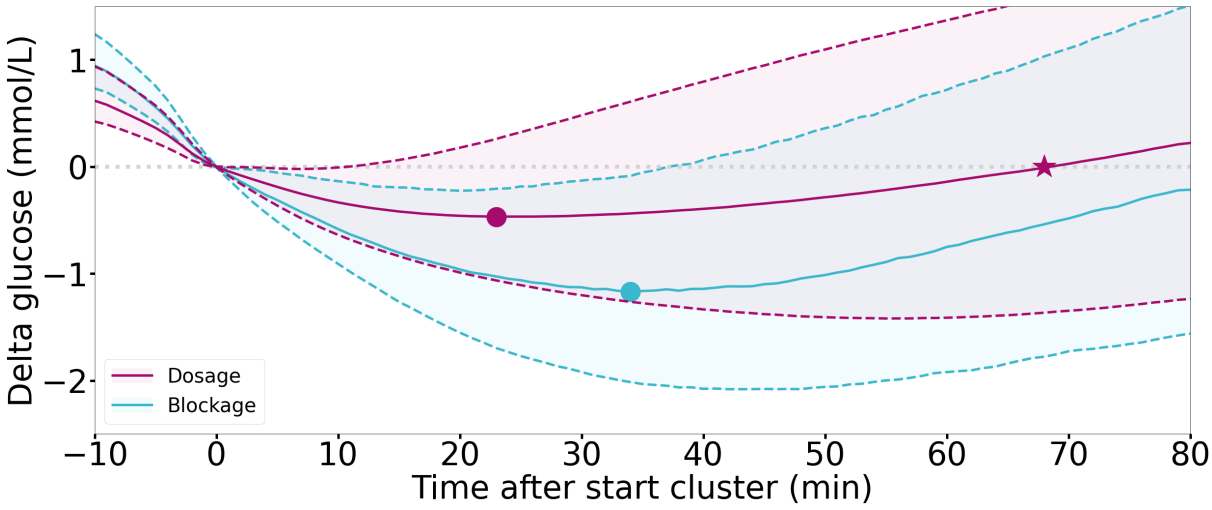


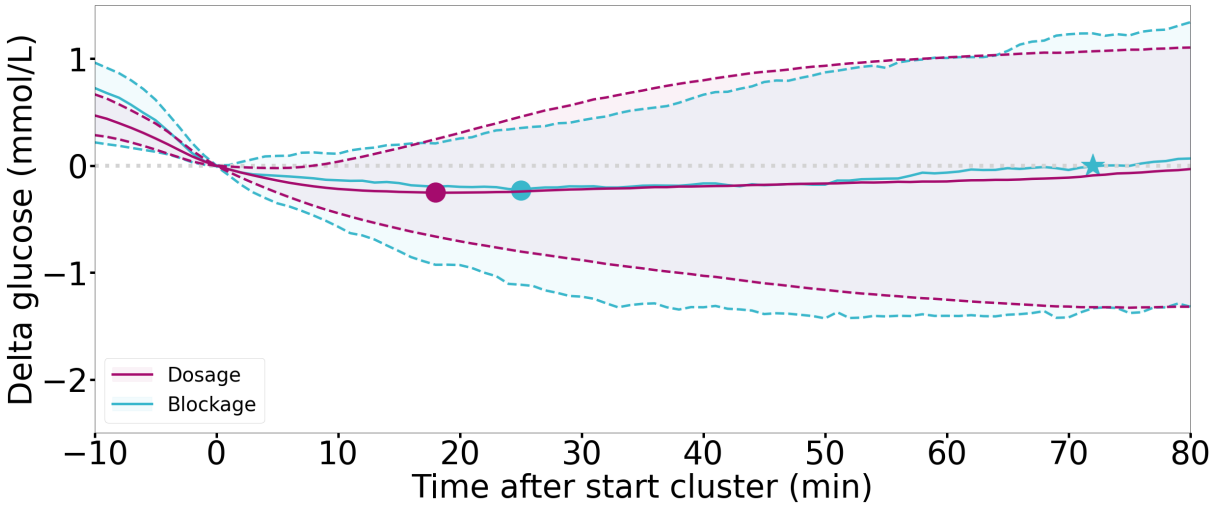
Figure 3.2: Flow diagram of data selection

3.3.2 Glucose response to glucagon

Figure 3.3 shows the difference in glucose changes after glucagon dosages and without glucagon dosage. The glucose levels steadily decreased before glucagon dosing, followed by a slower decline in glucose levels and after some time the glucose levels increased. As illustrated in Figure 3.3a, the decrease in glucose levels is less pronounced when glucagon is administered compared to when it is not. The endpoints are given in table 3.2. On average, the glucose levels rose 11 and 7 minutes earlier, and decreased 0.72 and -0.03 mmol/L less deeply, respectively, throughout the entire day and night. Maximum glucose values are always reached at the end of the observed timeframe. Throughout the entire day, the total area between the curve and the zero-excision line is three times larger in the presence of blockages than after successful glucagon dosages. During the night, the AUC only differs 4 mmol/L*h in both groups but are both smaller compared to the entire day. The deviation between the maximal drop in glucose levels of both groups is 0.24 mmol/L. The median slope before start of glucagon blockages clusters are more steep over the entire day and less steep in the night than glucagon dosages. The median dosage glucagon administered in one cluster is 8 units with an interquartile range of 2.5-14.5 units. For nighttime data, this is 7.5 [2.5-12] units of glucagon per cluster.



(a) Day



(b) Night

Figure 3.3: Glucose excursion after glucagon dosage and blockages A) throughout the entire day B) during the night. The solid line represent the median reaction, the shaded area between the dashed lines is the range between the first and third interquartile. • is the point where the maximal decrease in glucose is reached and ★ were baseline glucose is recovered.

Figure 3.4, shows a significant difference between glucagon dosage and blockages after the start but also ten minutes before. The maximum median difference between those groups is 0.77 mmol/L which is reached after 40 minutes. The spreading of delta glucose observed in the group of clusters receiving glucagon is larger than in the blockage group.

Table 3.2: Baseline characteristics and endpoints

	Entire day		Night	
	Dosage	Blockage	Dosage	Blockage
Baseline characteristics				
Number of events (#)	198.689	7.708	40.888	912
BGM_0 (mmol/L)	6.07	6.31	5.77	5.59
$Slope_{10\ min}$ (mmol/L/h)	-3.17	-4.77	-5.42	-2.70
Endpoints				
$AUC_{80\ min}$ (mmol/L*h)	-18.42	-62.52	-13.66	-9.65
AUC_{delta} (mmol/L*h)	-19.89	-	-	-9.83
G_{min} (mmol/L)	-0.45	-1.17	-0.26	-0.23
T_{min} (min)	23	34	18	25
$T_{delta\ =\ 0}$ (min)	67	-	-	72
G_{max}(mmol/L)	0.24	-0.22	-0.03	0.07
T_{max}(mmol/L)	80	80	80	80

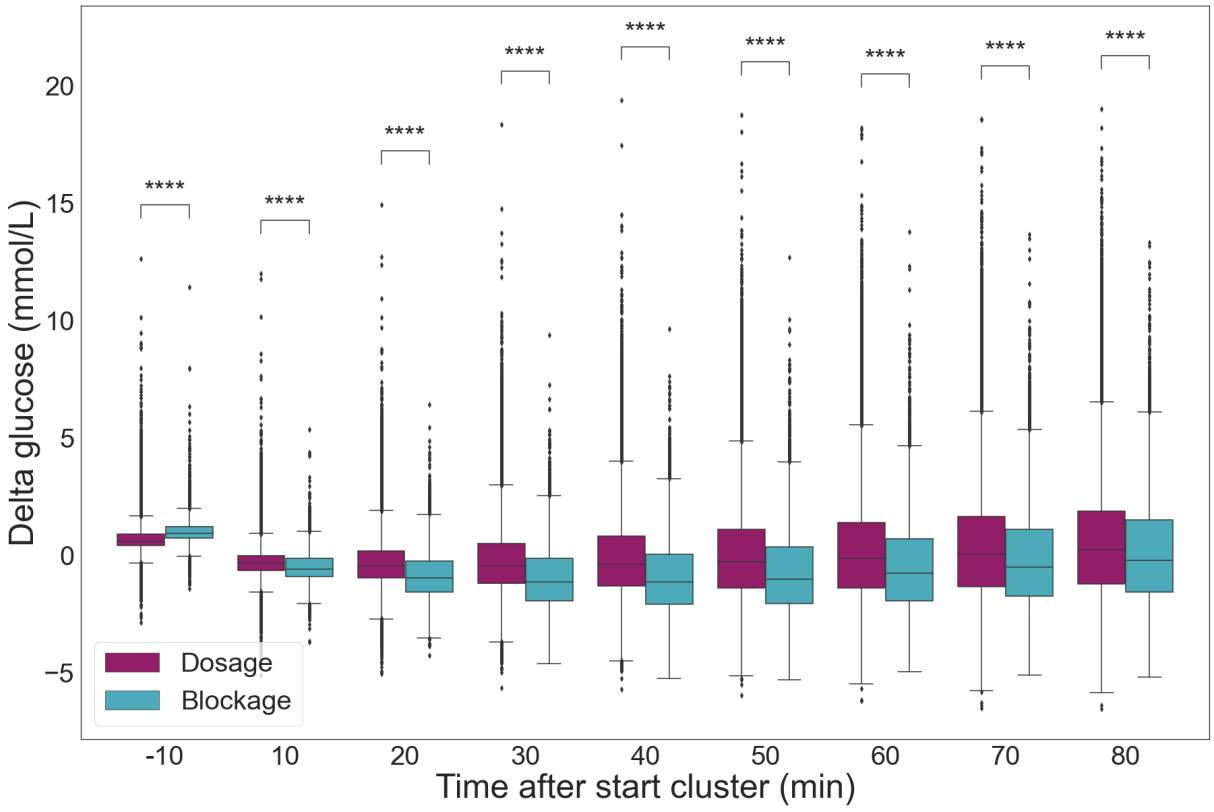


Figure 3.4: Boxplot analysis: dosage versus blockages

3.4 Discussion

Summary of findings

The results of the present study suggest that glucagon tends to let the glucose level decrease less and subsequently increase the glucose level quicker. In general, it ensures a 0.7 mmol/L less decrease and it takes 11 minutes less time to increase the glucose level in patient with type 1 diabetes. Nighttime data shows equal glucose trends by glucagon dosages and blockages.

Relation with literature
The finding that glucagon increase the blood glucose level within 80 minutes after dosing is consistent with studies which have assessed the glucose excursion after glucagon dosages. However, the effect size and effect time of glucagon on the glucose level deviated from those in the laboratory [4, 23]. Blauw et al. measured peak glucose concentrations in 6 patients after 40-80 minutes with glucose excursion ranging from 2.6 to 6.2 mmol/l. Ranjan et al. found glucose excursions between 2.7 and 5.3 mmol/L, with a peak concentration after 33-49 minutes of insulin induced hypoglycemia in 9 patients. The timing of peak glucose excursions aligns with our findings that the maximum deviation between doses and blockage clusters occurs around 40 minutes. Thereafter there is no relatively greater increase in glucose levels after administering glucagon compared to after a blockage. However, the glucose levels are still increasing after 80 minutes. They also found in their studies a dose dependency for peak glucose excursions and time to reach peak glucose. The difference in effect size of glucagon compared to those from literature can be assigned to different glucagon doses. In this study, on average 0.08 mg glucagon is administered in a single cluster while the smallest amount of glucagon in the studies were 0.1 mg glucagon.

Difference between day and night
One important observation is the significant difference between dosage and blockage during the day, while there is a lesser distinction at night between dosage and blockage. The primary reason for this difference is the glucose trajectory following a glucagon blockage during the night and day. A potential explanation for a more pronounced decline in glucose levels during the day, compared to the night, could be the occurrence of insulin-induced hypoglycemia following meals. Generally, glucose levels are more stable at night, resulting in a less significant decrease. Therefore, it seems that glucagon is less important in the night to remain in the target area. However, dosages of glucagon to maintain target glucose levels does not need attention of the patient and as a result less interruptions of sleep.

Difference between blockage and dosage
Despite the expectation that situations involving glucagon administration and blockage should be similar, a notable distinction is observed. The slope before glucagon administration deviates between dosage and blockages clusters. It appears plausible that this disparity could be linked to an increased likelihood of blockages when larger volumes of glucagon are indicated by the AP system. Larger volumes of glucagon are given when the glucose levels are decreasing fast. Hence, the scenarios under consideration lack complete comparability.

Strength and limitations
The challenges of directly translating results from controlled study settings to real-life scenarios are stated [4]. The main strength of the present study is that a closed-loop system was compared with an open-loop system, so that the effect of glucagon in real world data could have been assessed. A limitation of assessing the glucose raising effect of glucagon in a closed-loop system is that contextual knowledge lacks. Consequently, attributing the rise in glucose levels solely to the effect of glucagon becomes uncertain and can also be due to meals or other blood glucose raising factors. Besides the lack of contextual knowledge, the known dose-dependency of glucagon is not taken into account in this study as clusters with several dosages on different times are used. Therefore, generalizing to individual events is challenging, as this analysis only examines average responses in clusters.

Conclusions and further research
In conclusion, this study successfully achieved its objective of assessing and quantifying the impact of glucagon administration on glucose levels. Glucagon exhibits a 11 minutes earlier

rise and 0.7 mmol/L less drop in glucose levels in the Inreda AP compared to when it is not administered. Additional research is needed to identify factors influencing the effect size of glucagon to develop a full picture of implementation of glucagon in bihormonal closed-loop system.

4 Interaction of Glucagon and Insulin on glycemic control

4.1 Objective

This chapter examines whether the presence of insulin influences the impact of glucagon on glucose levels. Results of this study may yield valuable insights for enhancing the diabetes management with bihormonal closed-loop systems, potentially offering fundamental insights into physiological principles of glucose homeostasis.

4.2 Methods

4.2.1 Dataset

The dataset used in this study closely mirrors that of the previous study (Chapter 3.2.3), utilizing similar participants, dataset and a comparable data preprocessing. Differences are sorting the clusters.

Because, within the scope of this study, an analysis was conducted to compare the glucose excursion effect of glucagon in the presence and absence of insulin on board. Hence, the clusters were sorted into categories depending on whether insulin was given in the previous 60 minutes or if no insulin was injected in that 60 minute time frame. Maintenance dosages of insulin were excluded even as cluster with blockages at the start. The duration of Lispro insulin activity can extend up to 5 hours; however, in this context, the consideration specifically involved the peak activity, recognized for its immediate and predominant effect [20, 22].

4.2.2 Data analysis

First of all, the general effect of glucagon on the glucose patterns in the presence and absence of insulin was assessed through comparing the median-IQR plots and the endpoints. Secondly, the slope of glucose curve previous to the glucagon dosage (preceding slope) might influence the size of the glucose excursion after glucagon dosage. Insulin on board might be a predictor of this preceding slope. Therefore, clusters with similar preceding slopes are categorized to correct for this factor. Preceding slopes were calculated by averaging the slopes given by the AP in the ten minutes before the start of the clusters. The slopes examined in this study represent various rates of decrease: those below -10 mmol/L/h are considered extremely fast decreasing, between -8 and -6 very fast, between -6 and -4 fast, between -4 and -2 slightly decreasing, and from -2 to 0 mmol/L/h very slightly decreasing. Moreover, per slope category, the distribution of administered insulin and baseline glucose level were determined. All mentioned analyses were performed for the entire day and nighttime (00:00- 06:00 hours).

4.3 Results

4.3.1 Dataset

The number of clusters included is 72.949 for the absence of insulin on baord and 125.740 for the presence of insulin on board. The amount of insulin administered in the past hour before glucagon dosages ranges between 0.25 and 43 units with a median [IQR] of 1.75 units [1.00-3.00].

Table 4.1: Baseline characteristics and endpoints

	Entire day		Night	
	Insulin	No Insulin	Insulin	No Insulin
Baseline characteristics				
Number of events (#)	125.736	72.947	20.210	20.678
BGM_0 (mmol/L)	6.48	5.37	6.44	5.12
$Slope_{10\ min}$ (mmol/L/h)	-3.78	-2.12	-2.96	-1.55
Endpoints				
$AUC_{80\ min}$ (mmol/L*h)	-42.16	-18.42	-50.76	-13.66
AUC_{delta} (mmol/L*h)	-	-19.89	-	-
G_{min} (mmol/L)	-0.70	-0.45	-0.86	-0.26
T_{min} (min)	36	23	70	18
$T_{delta\ =\ 0}$ (min)	-	67	-	-
G_{max}(mmol/L)	-0.25	0.24	-0.30	-0.03

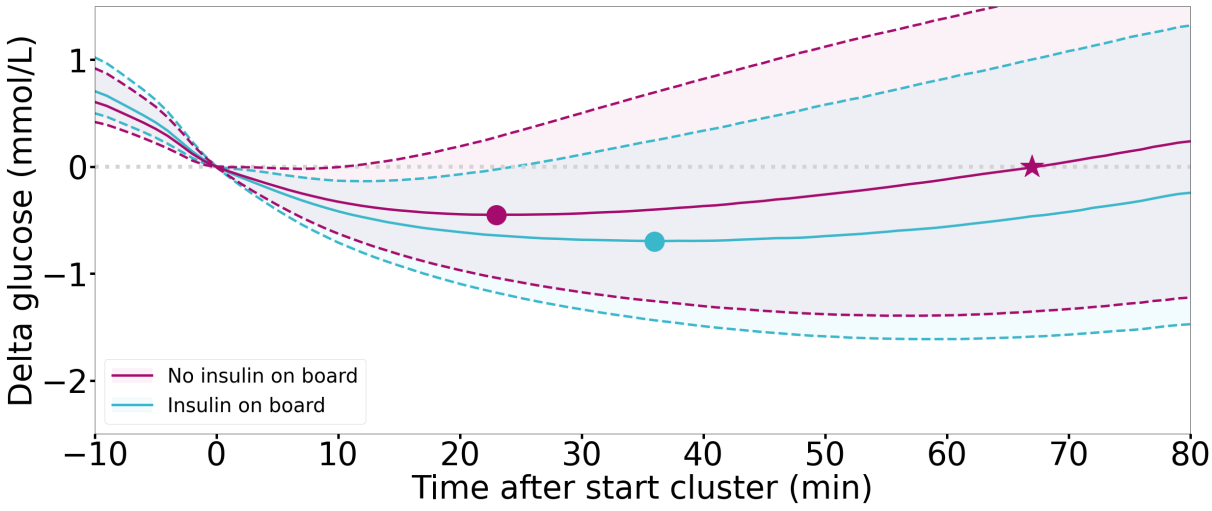
4.3.2 General finding

Figure 4.1 shows the glucose excursion after glucagon injection with and without insulin on board. During the entire day, glucose levels decrease by an average of -0.25 mmol/L less, increase approximately 13 minutes earlier and 0.49 mmol/L more within the first 80 minutes when insulin is not administered. At night, the decrease is around 0.40 mmol/L less, 52 minutes faster and 0.33 mmol/L more increase. In table 4.1, the endpoints are shown. The distinction between clusters occurring after insulin administration and those without is notably more evident in the nighttime data.

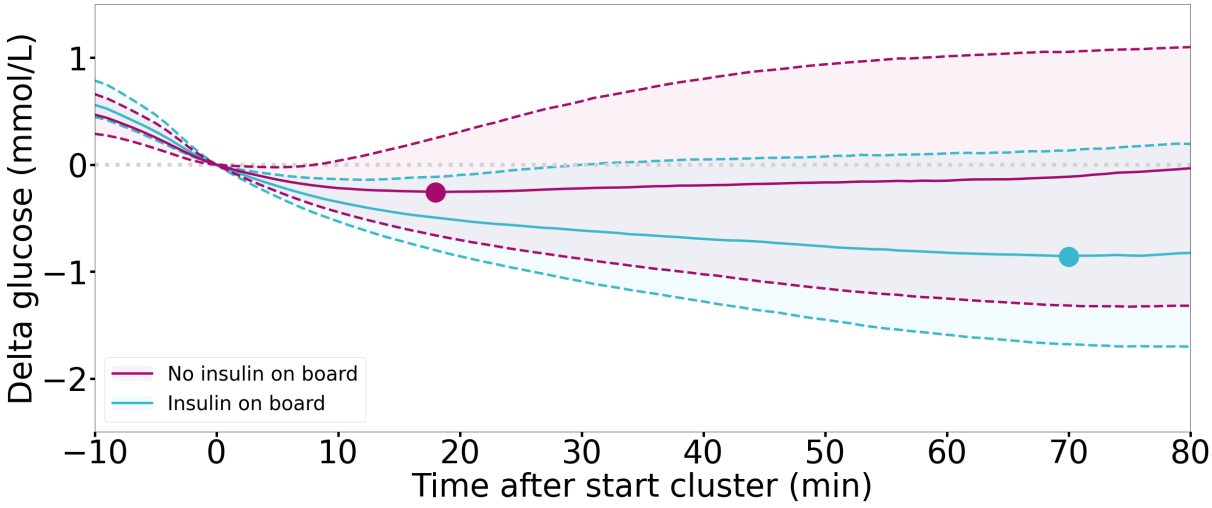
4.3.3 Slope analysis

Figure 4.2 shows the glucose response to glucagon dosage with and without insulin administration in the past hour shown for several slope categories. It stands out that the glucose drops longer, more and rises less when the slope is decreasing faster, regardless of the presence or absence of insulin on board. However, difference between insulin on board and no insulin on board is only observed in the slightly and very slightly decreasing slope categories. Furthermore, the glucose drops also longer and faster with insulin on board. In spite of, the fast decreasing category show slightly opposing results as the glucose levels of the without insulin on board group drops about 10 minutes longer than the insulin on board group.

As hypothesized, the insulin amount distributions shows that more insulin is on board by the faster decreasing slopes. Yet, no correlation was seen between slope and amount of insulin on board (see Appendix B.3). However, the distribution of baseline glucose values are similar for cluster with and without insulin on board, except for the first two slowly decreasing slopes, where the most difference in glucose response is observed. In Appendix B.4 the plots are shown for nighttime data only.

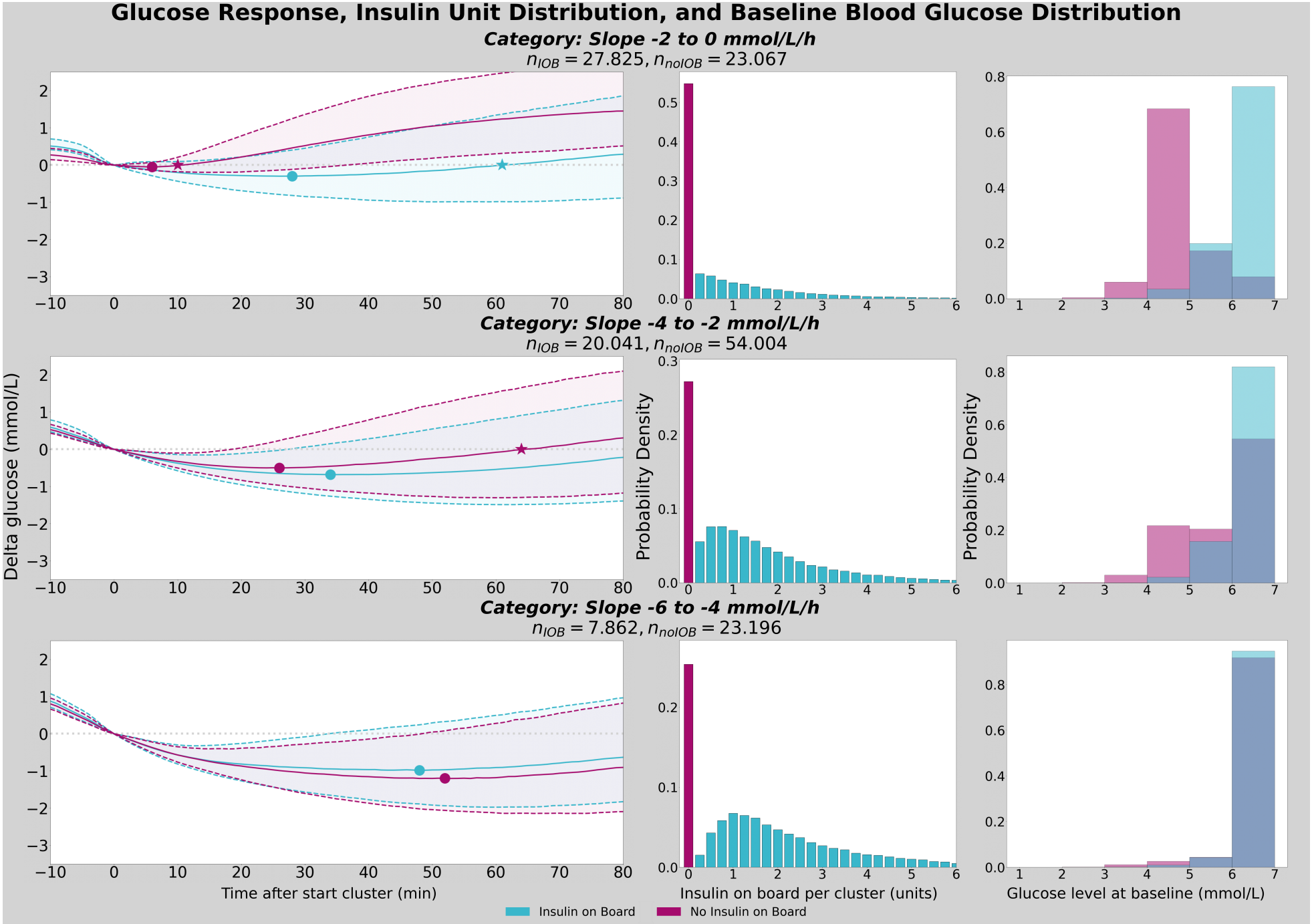


(a) Day



(b) Night

Figure 4.1: Glucose excursion with and without insulin on board A) throughout the entire day B) during the night. The solid line represent the median reaction, the shaded area between the dashed lines is the range between the first and third interquartile. • is the point where the maximal decrease in glucose is reached and ★ were baseline glucose is recovered.


Glucose Response, Insulin Unit Distribution, and Baseline Blood Glucose Distribution
Category: Slope -2 to 0 mmol/L/h
$n_{IOB} = 27.825, n_{noIOB} = 23.067$
Category: Slope -4 to -2 mmol/L/h
$n_{IOB} = 20.041, n_{noIOB} = 54.004$
Category: Slope -6 to -4 mmol/L/h
$n_{IOB} = 7.862, n_{noIOB} = 23.196$
Delta glucose (mmol/L)
Probability Density
Time after start cluster (min)
Insulin on board per cluster (units)
Glucose level at baseline (mmol/L)
Insulin on Board
No Insulin on Board

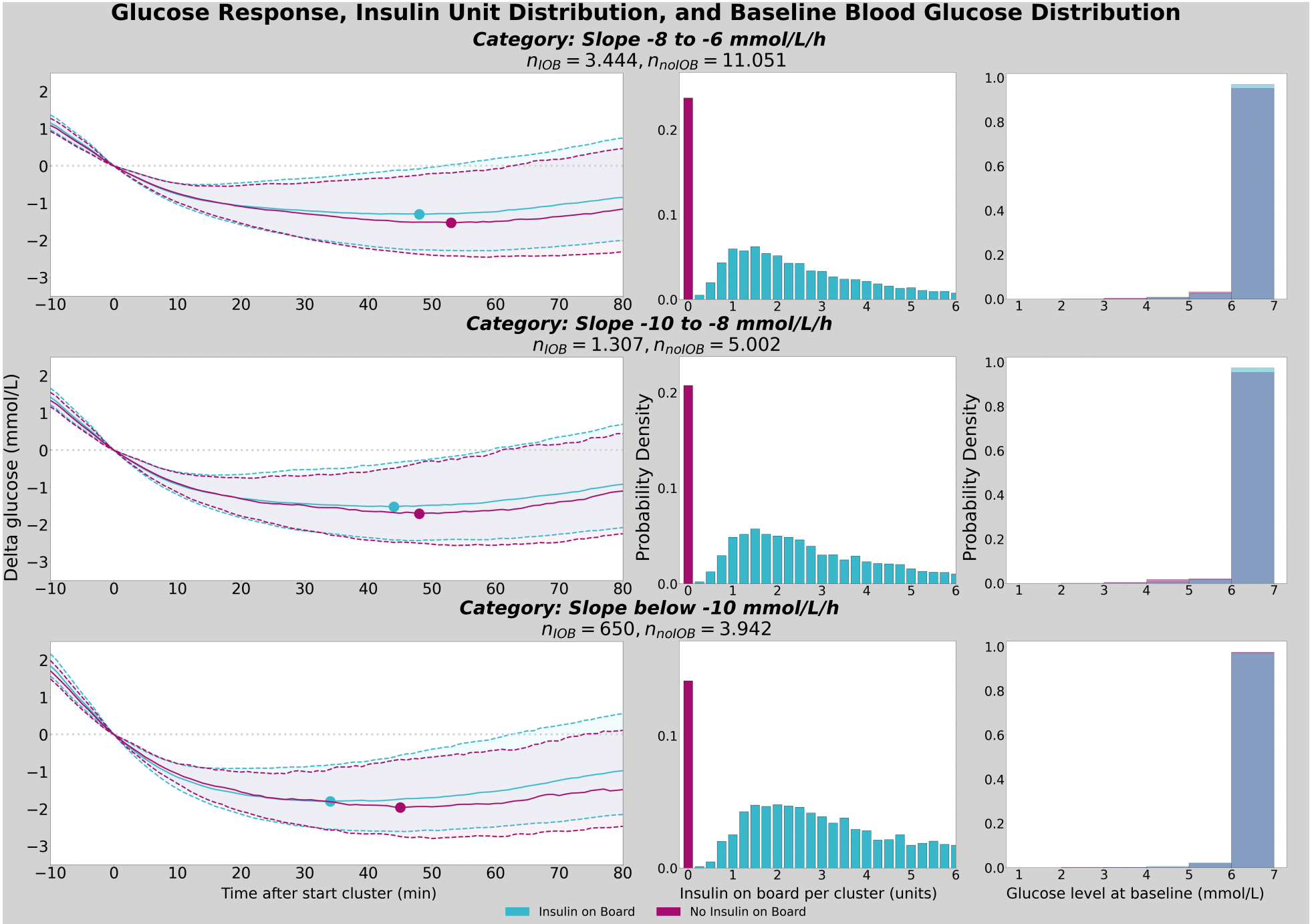

Glucose Response, Insulin Unit Distribution, and Baseline Blood Glucose Distribution
Category: Slope -8 to -6 mmol/L/h
$n_{IOB} = 3.444, n_{noIOB} = 11.051$
Category: Slope -10 to -8 mmol/L/h
$n_{IOB} = 1.307, n_{noIOB} = 5.002$
Category: Slope below -10 mmol/L/h
$n_{IOB} = 650, n_{noIOB} = 3.942$
Delta glucose (mmol/L)
Probability Density
Time after start cluster (min)
Insulin on board per cluster (units)
Glucose level at baseline (mmol/L)
Insulin on Board
No Insulin on Board

Figure 4.2: Slope analysis: Glucose Response, Insulin Unit Distribution, and Baseline Glucose Distribution. The rows represent the categories; clusters with similar preceding slopes in order of faster descending slopes. The first column, shows the glucose response after glucagon dosage with (IOB, blue) and without (no IOB, red) insulin on board. The solid line represent the median reaction, the shaded area between the dashed lines is the range between the first and third interquartile. • is the point where the maximal decrease in glucose is reached and ★ were baseline glucose is recovered. The second column, shows the distribution of total amount insulin administered in the past hour before glucagon dosage, with the y-axis representing probability density. The last column shows the distribution of baseline glucose values measured at the first glucagon dosage for IOB and no IOB group.

4.4 Discussion

Summary of findings

This study found that glucagon dosages with insulin on board reduces the impact of glucagon on the glucose response. Glucose levels show a 13 minutes longer and -0.25 mmol/L more pronounced decrease while also exhibiting a 0.49 mmol/L less increase within an 80-minute timeframe. At night, this difference is even more pronounced. In general, a more significant decrease in the slopes corresponds to a greater and more prolonged reduction in glucose levels regardless the presence or absence of insulin on board. Comparing clusters with insulin on board and no insulin on board, difference are visual at the lowest decreasing slopes and a slight opposing trend observed at the steepest declining slopes.

Relation with literature

Literature has already shown that insulin impact the response of glucagon [13, 27]. The glucose raising effect of glucagon is impaired by presence of high insulin levels. However, at moderate and low levels of plasma insulin, glucagon raises the glucose levels in a dose-dependent manner. In this study, the administered insulin doses over the past hour vary widely, ranging from 0.25 to 43 units. This variability likely extends to diverse levels of insulin plasma, as these are not measured in this study. Therefore, fully endorsing the findings observed in the academic literature is not feasible. However, the results of this study have shown that the glucose raising effect of glucagon is less when insulin is on board.

Slope analysis

Similar preceding slopes were categorized to be able to compare similar situations excluding effects of other factors. The difference in glucose response with and without insulin on board became less prominent as the preceding slope is steeper. In this sub-analysis, the slope is considered as the independent variable and observed glucose response difference as the outcome. This analysis reveals a slope-dependent effect whether or not insulin on board appears to influence the glucose response to glucagon. Hence, interpreting these findings is challenging due to the complex causality and additional confounding variables. First of all, the distribution of baseline glucose of the two least steep slope categories differs significantly between the two groups. Secondly, a relatively higher frequency of clusters with insulin on board in the steepest slope categories is observed. Steeper slopes, regardless of whether insulin is on board or not, already exhibit more significant declines in glucose levels compared to less steep slopes. This observation suggests that prioritizing the preceding slope might hold more significance than considering the administration of insulin in the past. Nevertheless, relatively more cluster with insulin on board and less without insulin on board where seen with steeper slopes.

Strength and limitations

The primary strength of this study lies in its utilization of real-world data, allowing for insights into the effects of insulin on board within dynamic settings. However, the inherent complexity of real-world data presents certain drawbacks, given the diverse and varied nature of each situation, resulting in difficulties to drawn conclusion about the cause - effect relations. Furthermore, insulin on board is taken into account as a binary variable rather than categorical or continues. The variability in insulin sensitivity and clearance across individuals and circumstances is not accounted for in this study and therefore lacks the possibility to generalize the results to individuals and situations. Lastly, a major disadvantage is not incorporating the amount of administered glucagon units, as it is known to have a dose-dependent effect on glucagon. Nevertheless, limited data is available at this moment to subdivide it on also the glucagon dosage.

Conclusions and further research

In conclusion, this study aimed to analyze the interaction between insulin on board and the effectiveness of glucagon. Glucagon dosage with insulin on board results in less glucose excursion on average 13 minutes longer and 0.25 mmol/L less maximal decrease. However, preceding slope seems to be more determined factor to the effect size of glucagon than the amount of insulin on board. To enhance the study's robustness, future research could explore the incorporation of variables such as insulin sensitivity and clearance, or alternatively, consider using plasma insulin levels to provide a more nuanced approximation of insulin on board. This adjustments has the potential to facilitate comparisons with existing literature, often based on fixed settings. Additional research is needed to identify more factors explaining the effect size of glucagon to improve glucagon to develop a full picture which factors are important to consider to further develop the treatment with bihormonal closed-loop system.

5 Intra- and inter-variability in glucose response of glucagon

5.1 Objective

In the previous chapter the effect of insulin on board was evaluated. Yet, all contributing factors to the observed difference in and between individual glucose response to glucagon is not fully examined. Examining potential contributing factors could offer valuable insights for improving diabetes management with bihormonal closed-loop systems. This exploration may suggest the inclusion of additional parameters in the algorithm or the optimization of personal settings to enhance treatment goals.

5.2 Methods

5.2.1 Dataset

The methodology employed in this study closely mirrors that of chapter 3, utilizing the identical participants, dataset and data preprocessing. The clustering of glucagon dosages was applied as mentioned in Chapter 3.2.2. For this study clusters starting with a glucagon dosages without blockage were included.

Additional data of the cluster were retrieved: Baseline glucose value (BGM_0), preceding slope ($slope_{10min}$), total units of glucagon given in a cluster (U_G). BGM_0 was calculated by the mean glucose level in a two-minute window, one minute before and one minute after the start of the cluster, in order to reduce impact of sensor errors (as discussed in Chapter 2.2.1) . $Slope_{10min}$ was retrieved from averaging all the measured slopes in the 10 minutes before the first dosage. U_G was the sum of all glucagon dosages in the cluster except for the anti-blockage dosages.

In addition, the age and gender were known for all patients. Additional patient characteristics were included from a specific subset of patients, as this data was not available for all patients. These patient characteristics were diabetes duration at start inclusion date, HbA1c before start AP treatment and total daily dosage insulin before start AP treatment ($TDDi_{bAP}$). Moreover, for all patients the average TDDi and the average insulin curve and VI setting over the study period were calculated.

5.2.2 Data analysis

Analyses were performed for inter- and intra- variability factors, which refer to the factors influencing the glucose response to glucagon within an individual (intra-variability) and between individuals (inter-variability). All analyses were also carried out using only nighttime data, with clusters initiated between 00:00 and 06:00 hours, in order to exclude the impact of meals and exercise. Table 5.1 shows the included factors and categories the clusters were divided into. Categories were based on reasonable intervals and ensuring that at least 5 patients were included per category to ensure a comprehensive and representative sample. Of all clusters, the delta glucose values were calculated each minute over a timeframe from 10 minutes before until 80 minutes after first glucagon dosage. For the inter-variability factors, clusters were grouped per category and averaged per minute using the median. Intra-variability factors were assessed by initially calculating the median average within a patient and subsequently calculating the median for each category. This two consecutive steps of averaging was applied in order to prevent patients with higher number of clusters from disproportionately influencing the

Variables		Measure	Categories
Inter-variability			
BGM_0	Baseline glucose measurement	mmol/L	[<3, 3.0-3.9, 4.0-4.9, 5.0-5.9, $\geq$ 6.0]
$Slope_{10min}$	Average of 10 minutes preceeding slope	mmol/L/h	[-2 to 0, -4 to -2, -6 to -4, -8 to -6, -8 to -10, $\leq$ -10]
U_G	Amount of glucagon injected in cluster	units	[<12,12-22,23-33, 34-44, >44]
Intra-variability			
Gender	-	-	[male, female]
Age	Age at start inclusion study	years	[20-29, 30-39, 40-49, 50-59, $\geq$ 60]
BMI	body mass index at start AP treatment	kg/m^2	[<20, 20-24.9, 25-29.9, $\geq$ 30]
$Dia_{duration}$	Time diagnosed with diabetes at start AP treatment	years	[<10, 10-19, 20-29, 30-39, $\geq$ 40]
HbA1c	HbA1c measured before start AP treatment	mmol/mol	[<48, 49-53, 54-64, 65-75, $\geq$ 76]
TDD_{bAP}	Total daily insulin dosage before AP treatment	units	[<40, 40-59, 60-79, 80-99, $\geq$ 100]
TDD_{AP}	Averaged total daily insulin dosage during study	units	[<40, 40-59, 60-79, 80-99, $\geq$ 100]
I_{curve}	Average Insulin curve setting	%	[<25, 25-50, 50-75, 75-100, >100]
I_{VI}	Average Insulin VI setting	%	[<2.5, 2.5-5.0, 5.0-7.5, 7.5-10.0, >10]

Table 5.1: Factors included in this study including definition, unit of measure and categories clusters were divided in.

results. The starting point for understanding the variations in glucose response was the differences between the categories. This assessment relied on visually inspection of the differences between categories as depicted in the median delta glucose response plots.

5.3 Results

5.3.1 Dataset

In Table 5.2 patient characteristics are shown. Of the patients, 65 are male, 63 are female and for one patient it was unknown. What stands out is that the insulin curve setting, insulin VI setting and the TDD varies significantly, as women seems to be more insulin sensitive than

	Male	Female
N	65 (*36)	63 (*34)
I_{curve}	80 [57 - 111]	44 [33 - 70]
I_{VI}	3.75 [3.00-5.75]	2.75 [1.75 - 3.75]
Age	49 [36 -55]	49 [41 - 56]
Diabetes duration*	26 [18 - 39]	26 [14.5 - 39.5]
HbA1c*	60 [55 - 68]	61 [54 - 67]
$TDDi_{bAP}$*	50 [38 - 72]	35 [30-51]

Table 5.2: Patient characteristics

male patients.

5.3.2 Inter-variability

BGM_0

Figure 5.1 shows the trajectory of glucose levels after glucagon dosage at various baseline glucose values. What stands out from the figure is the inverse relationship between baseline glucose level and the subsequent rate of change and magnitude of glucose excursion. Specifically, lower baseline glucose level correlate with faster and more pronounced increase in glucose excursion. Glucagon dosages administered during the night shows a less pronounced response to glucagon than during day-time. At glucose levels below 3 mmol/L, this difference is not visible in maximum glucose excursion but the trajectory differs. Additionally, it is noteworthy that the preceding slope varies across the distinct baseline glucose categories.

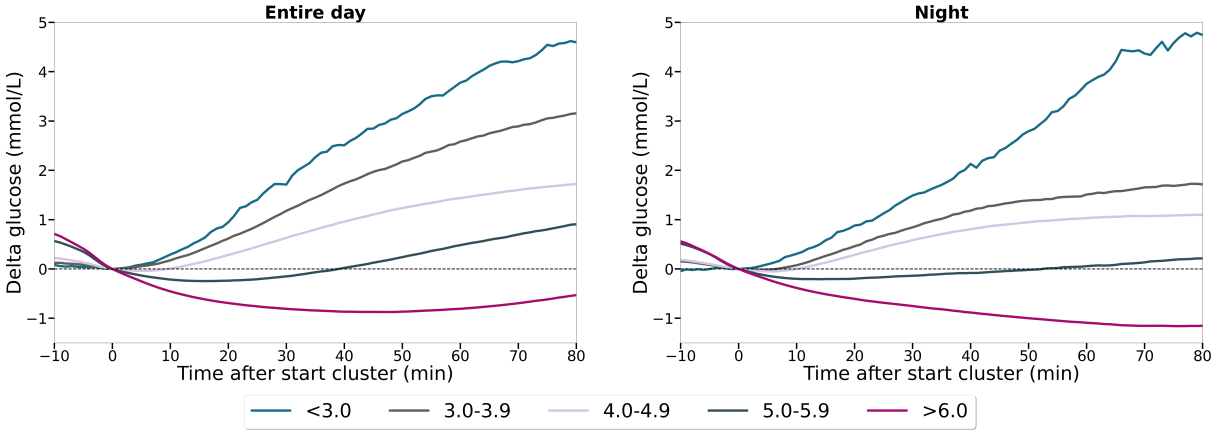


Figure 5.1: **BGM_0**: Inter variability in glucose response among various baseline glucose measures. Numbers of clusters during the entire day (n_d) and during night only (n_n). [<3.0] n_d = 295, n_n = 79; [3.0-3.9] n_d = 5.039, n_n = 1.174; [4.0-4.9] n_d = 34.153, n_n = 12.439; [5.0-5.9] n_d = 25.895, n_n = 5.517; [>6.0] n_d = 133.300, n_n = 21.679

$Slope_{10min}$

Figure 5.2 shows the glucose excursion for clusters with different rates of decrease in glucose levels before glucagon dosage. As the glucose levels decreasing faster, the glucose level drops lower, takes longer to increase and will increase less. The maximum glucose excursions is reached after 80 minutes and exhibit equality among the fastest three decreasing slopes categories. During the night, the glucose levels deviate after about 10 minutes from the trajectory seen during the entire day. The glucose levels in the night drops longer and lower than during the day. Moreover during nighttime, in the three fastest decreasing categories an infliction point is visible around 25 minutes, where first the slope is decreasing and subsequently less decreasing or even slightly increasing following a further decrease.

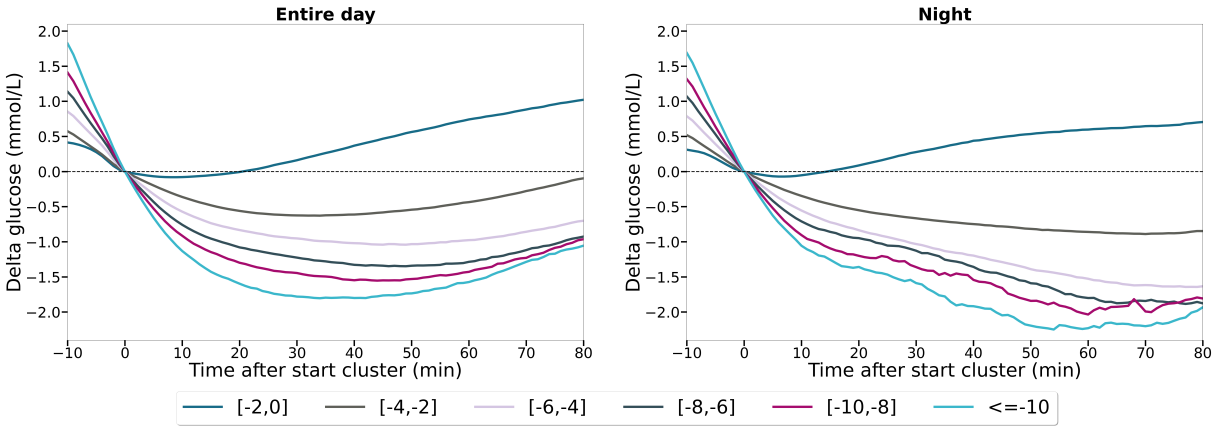


Figure 5.2: **Slope$_{10min}$**: Inter variability in glucose response among various preceding slopes (mmol/L/h). Numbers of clusters during the entire day (n_d) and during night only (n_n). [-2,0] n_d = 50.892, n_n = 16.370; [-4,-2] n_d = 74.045, n_n = 15.945; [-6,-4] n_d = 31.058, n_n = 3.613; [-8,-6] n_d = 14.495, n_n = 1.116; [-10,-8] n_d = 6.309, n_n = 338; [<=-10] n_d = 4.592, n_n = 175

U_g

Figure 5.3 illustrates the variability among clusters with varying amounts of glucagon dosage within each cluster. Throughout the day, the figure demonstrates that a lower requirement for glucagon corresponds to an earlier deviation from the decreasing slope. During the day the median glucose response shows an increasing slope within 80 minutes till 44 units and during the night till 33 units of glucagon. During the night 73% of the clusters return to BGM_0 within 80 minutes after one dosage, as there is no need for a second dosage of glucagon, compared with 67% during the day. At night, the decreasing slope deviates between all categories after 10 minutes and during the day it takes up till 50 minutes before the last two categories deviates from trajectory.

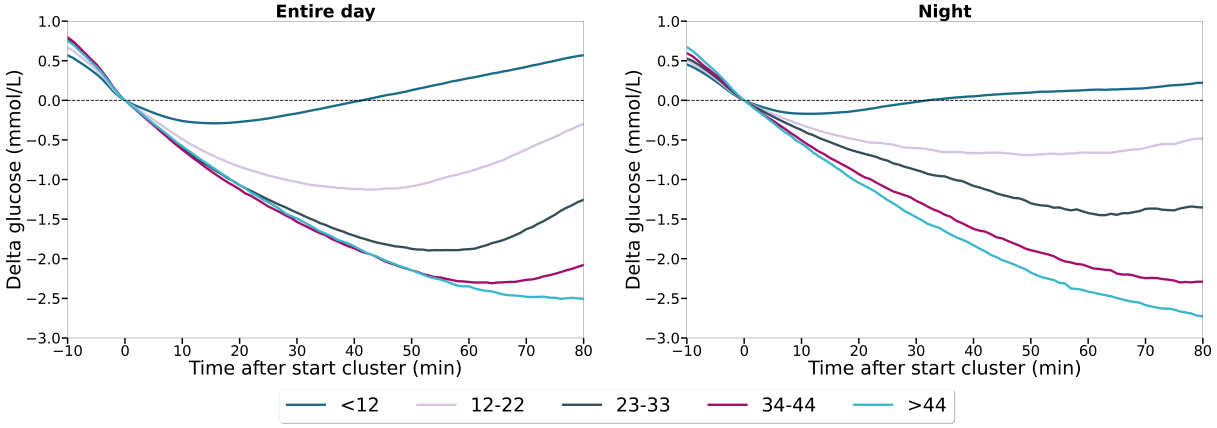


Figure 5.3: **U_g**: Inter variability in glucose response among various total glucagon dosages per cluster. Numbers of clusters during the entire day (n_d) and during night only (n_n). [<12] n_d = 133.504, n_n = 29.956; [12-22] n_d = 40.350, n_n = 6.449; [23-33] n_d = 15.192, n_n = 2.358; [34-44] n_d = 5.945, n_n = 1.188; [>44] n_d = 3.656, n_n = 934

5.3.3 Intra-variability

In all individual variable analyses, it is observed that the difference in glucose excursions between the categories varies less during the night than during the day.

Gender

Figure 5.4 shows the median glucose excursion of female and male patients. The time to maximum glucose-drop and the magnitude of the glucose-drop are equal for both groups. However, female patients show a faster increase afterwards in glucose levels during the day as well as during the night. Female patients achieve approximately 10 minutes earlier their BGM_0 than male patients.

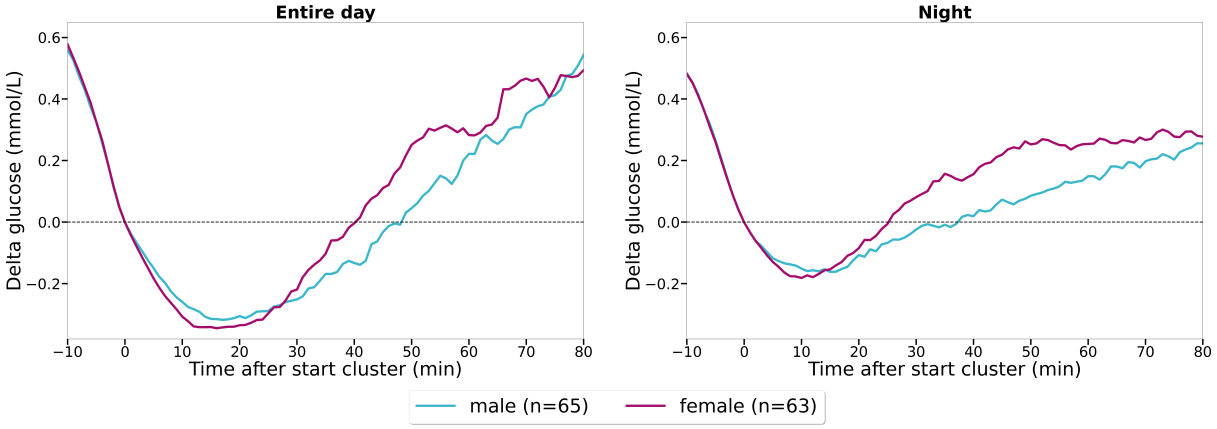


Figure 5.4: **Gender**, median glucose response of female and male patients during the entire day and nighttime.

BMI

Figure 5.5 illustrates that glucose excursion after recovering baseline level correlates inversely with BMI during the night. Throughout the day, individuals classified as underweight or normal weight exhibit an increase of approximately 0.2 mmol/L more than their overweight counterparts.

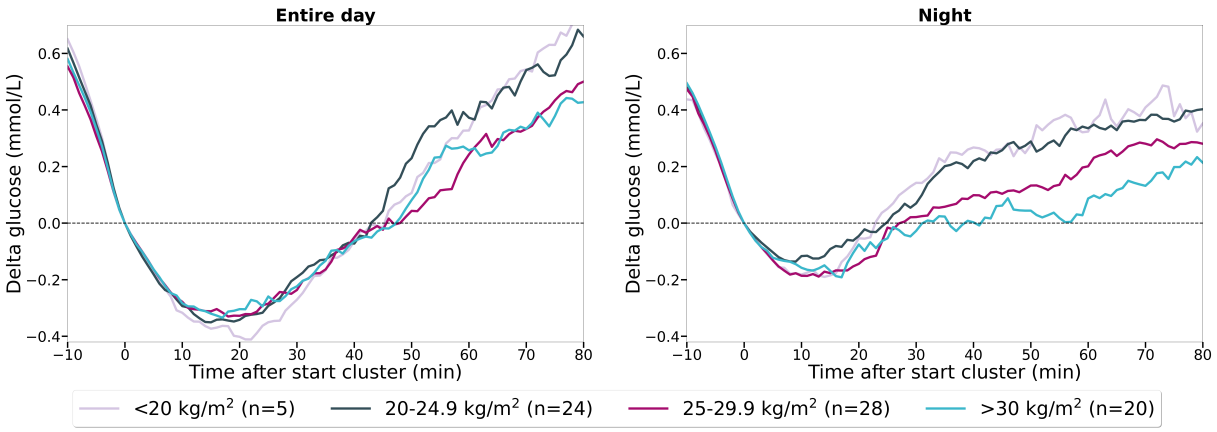


Figure 5.5: **BMI**, variability in median glucose response among patients with different BMI.

TDDi

Figure 5.6 shows the glucose trajectory of groups with various total daily insulin during the study. The different groups shows diverse glucose trajectories after comparable decrease in glucose. In general, patients who are using fewer insulin per day, show faster increase in glucose levels and more increase after glucagon dosage. During the night, the difference between patients using less than 40 units and their counterparts is even more pronounced. The total daily insulin dosage before AP ($TDDi_{bAP}$) treatment demonstrates a comparable response as during

AP treatment. The plot of variable $TDDi_{bAP}$ is shown in Appendix C.7.

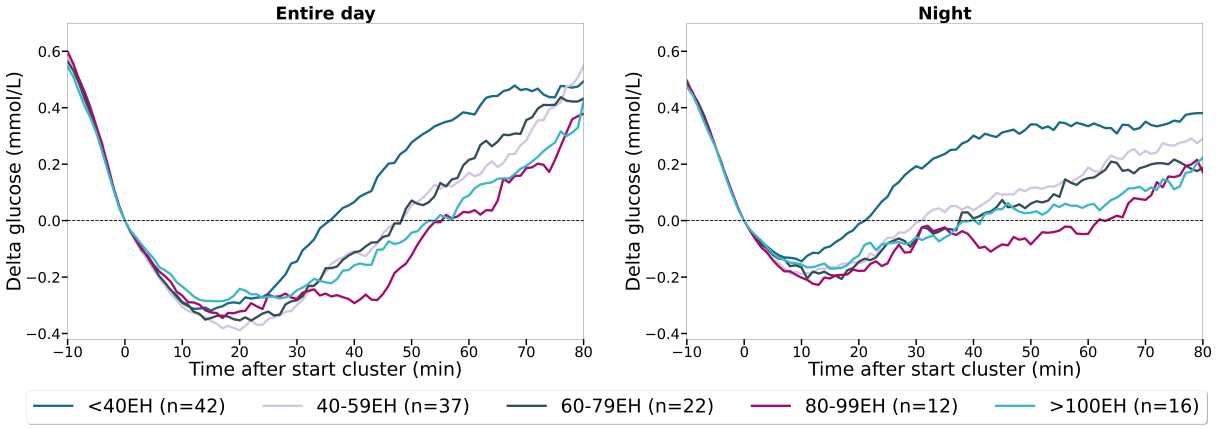


Figure 5.6: **$TDDi_{AP}$**, variability in median glucose response among various total daily insulin dosage during the study

Insulin settings

Figure 5.7 shows the variability in glucose response for different curve settings of insulin. Curve settings less than 25% shows a faster increase of glucose level during the day and during the night. It also shows a more significant increase in magnitude. VI settings shows less variability between the groups (see Appendix C.9).

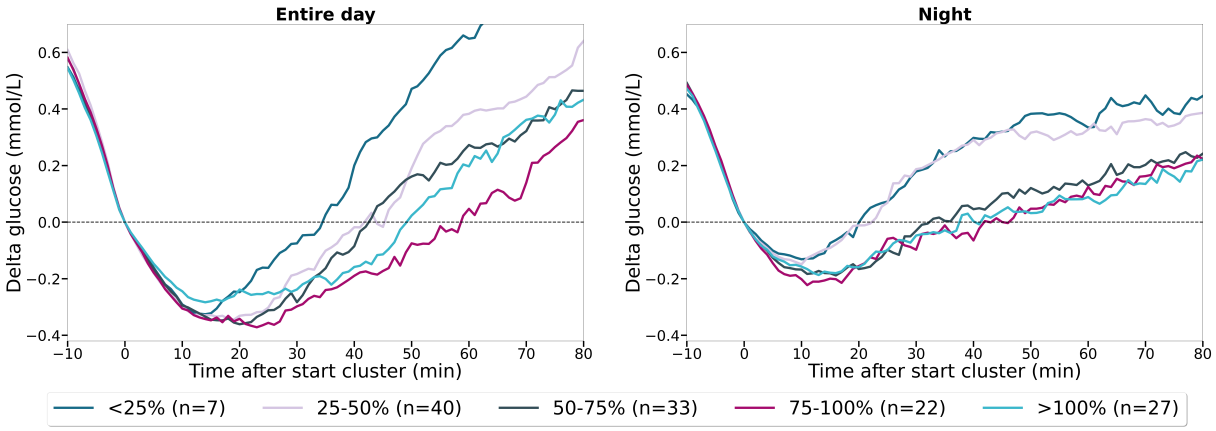


Figure 5.7: **I_{curve}**, variability in median glucose response among various insulin curve settings during the study

Other factors

The remaining analysis of potential contributing factors does not show clear correlations in glucose response. The figures can be found in Appendix C. Age shows no significant difference in glucose response in both entire day and only night data. Diabetes duration shows a 0.1 mmol/L more decrease during the entire day in the categories till 19 years of being diagnosed with Diabetes Type 1 compared to individuals with a longer diabetes duration. However, after 80 minutes the glucose excursion are consistent across all groups. A consistent trajectory is observed among various HbA1c values before AP treatment during the entire day. However, the maximum glucose excursion shows an inverse correlation with the magnitude of HbA1c. During the night, this correlation does not hold completely true; yet, individuals with HbA1c levels above 76 mmol/mol exhibit less glucose excursion compared to the other groups.

5.4 Discussion

Summary of findings
This study has investigated the contributing factors of the glucose response to glucagon. The inter-variability factors slope, baseline glucose level and amount of glucagon given are contributing to the glucose response. The intra-variability factor BMI has shown an inverse correlation with glucose response, where the factors gender, total daily insulin dosage, HbA1c and insulin curve setting showed an evident difference between a single group and the other groups. Moreover, the fluctuations in glucose are less significant during the night.

Relation with literature
Very little was found in the literature on the question of what the contributing factors are which affect the glucose raising effect of glucagon. Most study assessed the plasma level of endogenous produced glucagon in Type 1 diabetes during hypoglycemia instead of exogenous administered glucagon [18, 30]. In two study, the pharmacodynamics for various exogenous glucagon dosages were assessed by different levels of hypoglycaemia and non-hypoglycaemia levels [4, 15]. Blauw et al. found that higher blood glucose levels show less glucose excursions which is in line with the findings of this study [4]. The same study also revealed a correlation between higher glucagon dosages and increased glucose excursions. Hinshaw et al. also found a dose dependency but did not find a hepatic glucagon sensitivity difference between euglycemia and hypoglycemia [15]. However, it's important to note that in this study, clusters of glucagon dosages were employed within a closed-loop system. This system ensures that if a glucagon dosage is insufficient, another dosage will be administered. Therefore, no literature is found with comparable study design and no conclusions about the dosage dependency of glucose excursion can be draw from the results in this study.

Implications
The results of this study has multiple implications: First, the results showed that the effect of glucagon can vary between different preceding slope and baseline glucose values. This supports the importance of using these as input variables in the closed-loop algorithm. Although the dosages already depend on these factors, these results do not show if this is sufficient to prevent hypoglycemia. Second, repeated dosages of glucagon seem to work within 80 minutes of the first dosage till 44 units, more glucagon dosages does not stop the glucose from decreasing within 80 minutes. Nevertheless, it is unknown what happens when glucagon dosages were stopped earlier. Third, gender and BMI may serve as indicators of how well an individual responds to glucagon, alongside the insulin curve setting. Building upon the insulin curve as a measure of insulin sensitivity, it could indicate a correlation with individual glucagon sensitivity.

Strength and Limitations
This study applies individual variable analysis to explore causality between the factor and glucose response. This approach allows for a focused examination of the impact of a single variable on the outcome, improving our understanding of the relationship. However, while providing valuable insights, individual variable analysis may offer only a basic understanding of a complex system. The number of patients in this study was limited, which could lead to non-comparability of groups on confounding factors, thus affecting causal inference. Multivariate methods using more patients could offer a more comprehensive exploration, showing more details of dependency between variables and therefore a deeper understanding of its complexity.

Conclusions and further research
To conclude, the objective of this study was to evaluate potential factors, both within and between individuals, that play a significant role in inluencing the response to glucagon. The con-

tribution of several factor to variations seen in and between patients is assessed by individual variable analysis. Inter-variability factors BGM and slope contribute to the response to glucagon. Evaluating the impact of glucagon dosage is challenging in this study and as a result, no conclusions are drawn. Intra-variability factors as BMI, TDDi and insulin curve setting shows correlation with the glucose excursion, indicating a possible correlation between insulin and glucagon sensitivity. However, further research is needed to connect this gained knowledge and application in the AP treatment. In individuals, AP treatment could be adapted by changing glucagon or insulin settings. Research should be performed to investigate how these settings influence treatment.

6 Glucagon release in the bihormonal closed-loop system

6.1 Objective

In the past chapters the effect of glucagon on the glucose levels was examined under various conditions. However, the dosage of glucagon was determined by the AP algorithm. The impact of glucagon settings and how changes in these settings affect the AP treatment remain unknown. This study examines the influence of glucagon settings on glucose regulation. This may provide valuable knowledge to optimize settings in the AP treatment.

6.2 Methods

6.2.1 Dataset

This study utilized the dataset mentioned in chapter 3.2.1, which includes among others, continuous glucose measurements, time and amount of glucagon- and insulin dosages and personalized settings. Glucagon settings consists of curve, maximal correction injection (VI) and maximal extra injection (EI). Standard settings for glucagon were 11 units for VI and 8 units for EI and can only be adapted manually. Curve injections were excluded in the analysis because this setting does not differ among patients. Insulin settings only consist of curve and VI settings and can be changed both manually and by self-learning algorithm of the AP.

6.2.2 Data analysis

To assess the impact of glucagon settings on glucose regulation, periods of consecutive days without any adjustments in glucagon settings were identified and extracted for every patient, defined as periods. Periods of less than 4 weeks were excluded, to minimize the variability from short periods of time. Three forms of glucagon settings and three levels of glucose regulation with multiple outcome variable were defined, so nine combinations were formed (see Figure 6.1).

Glucagon settings were assessed on three levels:

1. Absolute settings: periods of all patients with same glucagon VI or EI were grouped and compared with other settings.
2. Ratio between glucagon and insulin settings: periods with different glucagon and insulin settings were compared to investigate the impact on glucose regulation. Categories were generated by combining different glucagon settings and insulin curve setting as measure for insulin sensitivity. Glucagon settings included standard (VI 11 EH and EI 8 EH) or reduced glucagon (lowered EI/VI). Insulin settings varied across low, moderate, and high, determined by median insulin curve settings in that period: low (<25%), moderate (25%-50%), and high (>50%). These six combinations formed the basis for the ratio assessments.
3. Change in glucagon settings: participants were selected by whom a glucagon setting was changed within the study period. Difference in periods before and after the change were compared between lowered setting, increased settings and a reference group. The

reference group comprises individuals without any change in glucagon setting during the study period, and the deviation is calculated from the group median of these participants.

Categories at the three levels of glucagon settings were compared on outcome measures at three different levels related to glucose regulation:

1. Overall management: parameters of overall glucose management were Time in Range (TIR), Time Above Range (TAR) and Time Below Range (TBR). First, the median glucose of every 5 minutes was calculated to reduce the amount of data in order to stay within the limits of computational capacity. Thereafter, the percentage of time with glucose values below 3.9 mmol/L, between 3.9 and 10.0 mmol/L, and above 10 mmol/L corresponding to TBR, TIR, and TAR, respectively, was calculated for each period of identical setting settings per patient.
2. Hormone usage: parameters were median daily dosage insulin (TDDi) and glucagon (TDDg). Moreover, the median amount and number of injection per kind were also assessed.
3. Clusters: parameters based on the cluster defined in Chapter 3.2.2 were median number of clusters per day, median cluster duration (time between first and last dosage) and median amount of glucagon in cluster.

Boxplots were generated for each outcome parameter to facilitate visual comparison among the categories.

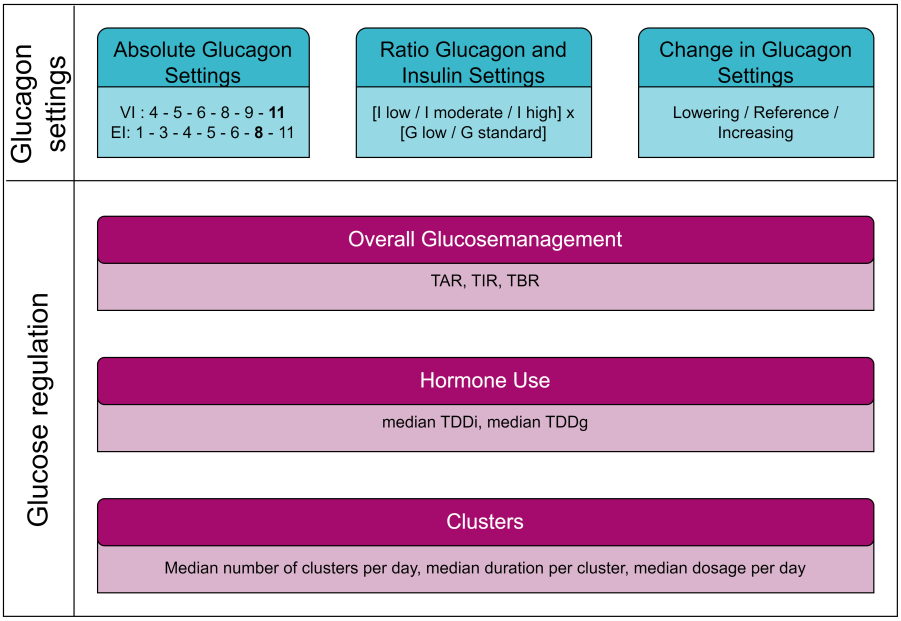


Figure 6.1: Overview of method. The blue boxes highlight three forms of glucagon settings - absolute value, glucagon/insulin setting, and setting changes. The light blue boxes show the categories which are compared. Pink boxes indicate the levels of glucose regulation, with light pink boxes illustrating specific outcome parameters against which the categories are compared.

6.2.3 Statistical analysis

Statistical tests were performed on all analyses. Non-parametric Kruskal Walis test was carried out to analyze absolute glucagon settings and determine if there were any significant differences among the glucagon settings. Mann-Whitney U-test was performed by analysis of ratio's

and change in glucagon setting. The groups were compared between custom and standardized settings within similar insulin settings. Effect of changing glucagon setting, both lowering and increasing, was only compared with the reference group. P-values <0.05 was considered significant different.

6.3 Results

6.3.1 Dataset

259 periods are identified of which 25 periods with a duration of less than 4 weeks are excluded. The periods have a median duration of 153 days [IQR: 104-266 days]. Thirty-five percent of the periods consist of standard glucagon settings. A total of 107 adaptions in glucagon settings were made within a year, comprising 48 reductions and 59 increases. Noted, 21 of these increases in glucagon settings are due to adaptions by a software update.

6.3.2 Absolute glucagon setting

Comparing various glucagon settings showed us the following: Firstly, analysis of distribution of TAR, TIR and TBR for different glucagon VI and EI settings has not shown significantly differences (see Appendix D.10). Secondly, figure 6.2 shows the total mean glucagon dosage and insulin dosage per day by different glucagon VI and EI settings. TDDi is lower by custom VI and EI setting but no significant difference is observed for TDDg. Additional differentiation between mean daily dosage curve, VI and EI dosages and number of injections showed ambiguous results. Thirdly, figure 6.3 shows that, the number of clusters increases on average one cluster per day more by lower VI and EI injection than standard settings. The typical dosage administered within a cluster closely aligns with the VI setting, suggesting that the amount of a single VI is usually given in a cluster and likely sufficient most of the time. As most clusters only contains a single VI and therefore the duration of cluster is zero minutes, it takes longer for the other clusters to restore the desired glucose target. A maximal VI setting of 6 units showed a significant increase than the other settings even as a maximal EI setting of 4 and 1 units.

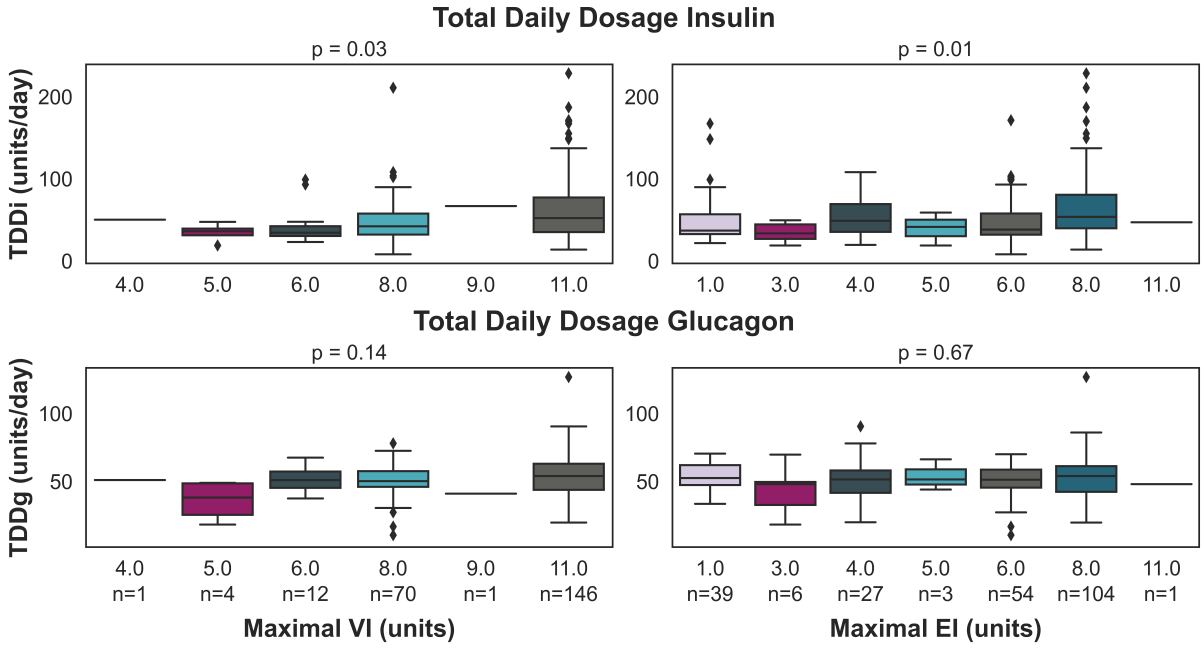


Figure 6.2: Hormone usage per glucagon setting. N = number of periods.

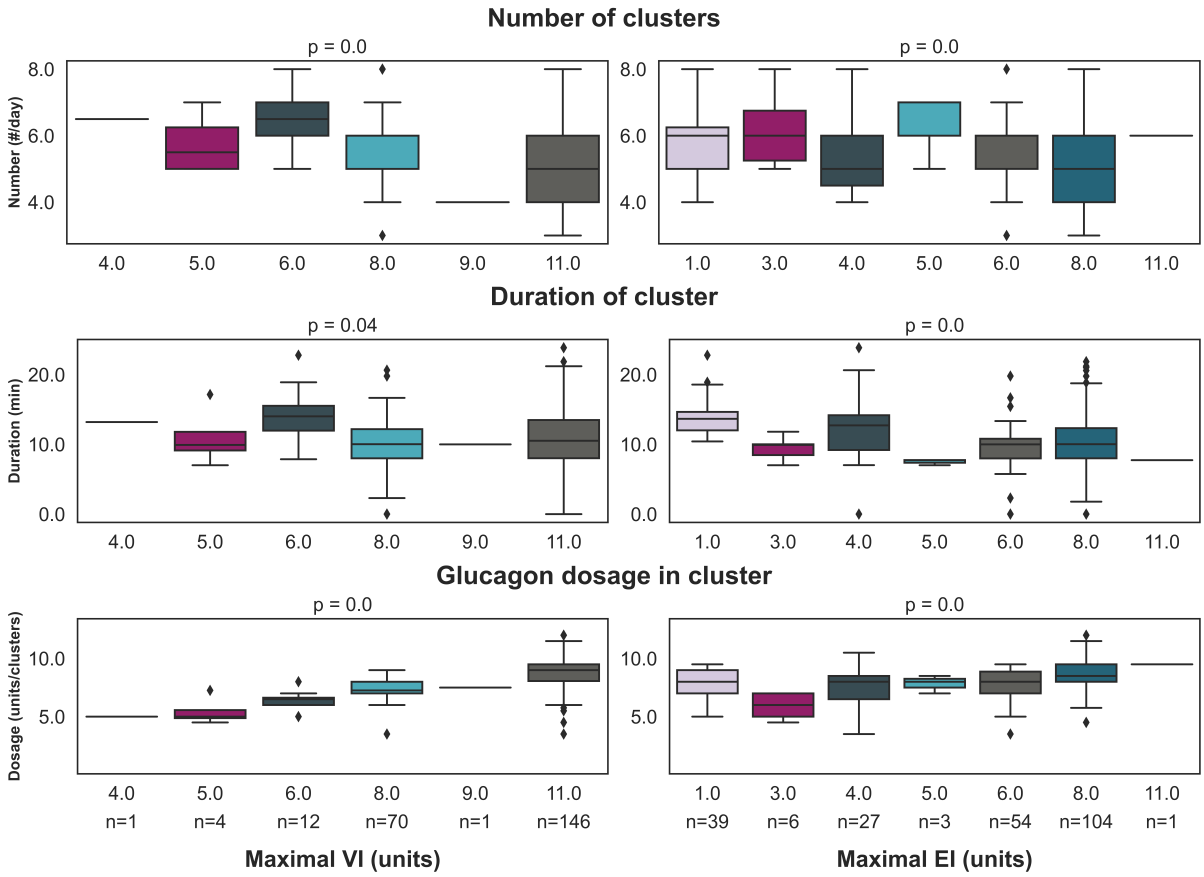


Figure 6.3: Cluster parameters per glucagon setting. N = number of periods.

6.3.3 Ratio of glucagon and insulin settings

Examining periods with varying ratios of glucagon and insulin settings reveals the following: First, Figure 6.4 shows the TIR, TAR and TBR for several combinations of glucagon and insulin settings. The high insulin curve category shows a significant difference between custom and standard glucagon setting in TBR. The periods with customized glucagon settings are longer below 3.9 mmol/L. No significant difference between the other categories or ranges. Second, figure 6.5 shows the hormone usage per category. In contract with the absolute settings, it showed no difference in TDDi between standardized and customized glucagon settings. Nevertheless, the TDDi correlates with the insulin curve, the greater the insulin curve setting, the more insulin use. The median glucagon usage varies within 5 units per day for each category. Only the moderate insulin curve settings shows significantly less glucagon use by custom settings. Third, figure 6.6 explains that glucagon dosages remain relatively consistent across all categories as the number of clusters increases, while the dosage within each cluster decreases for lower glucagon settings. Cluster durations do not differ significantly between standard and customized glucagon settings.

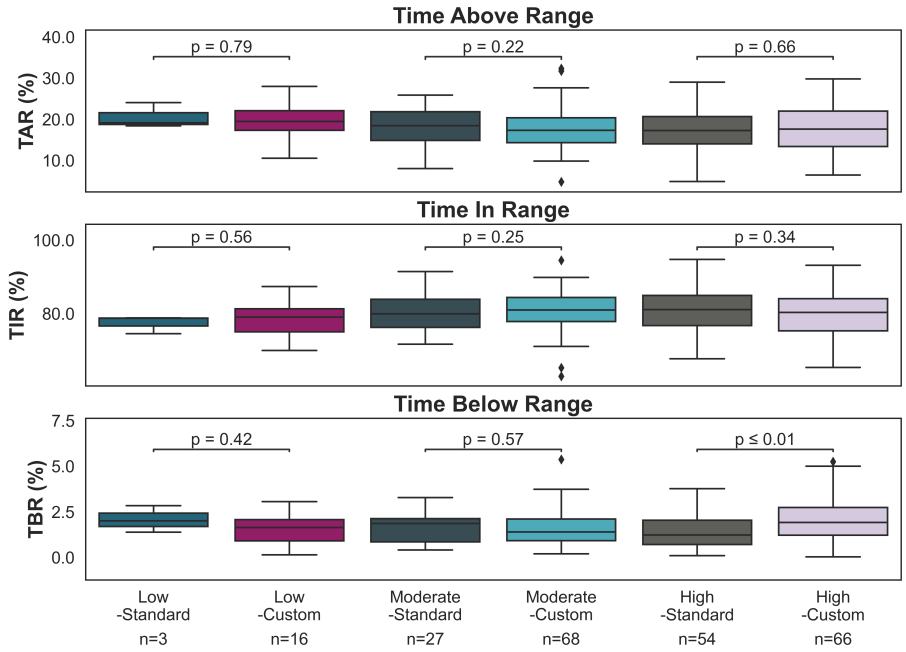


Figure 6.4: Overall glucose management per ratio between glucagon setting and insulin curve setting. Standard is default glucagon settings. Custom is adapted to lower VI and/or EI glucagon setting. Low <25% insulin curve, Moderate 25-50% and High >50%.

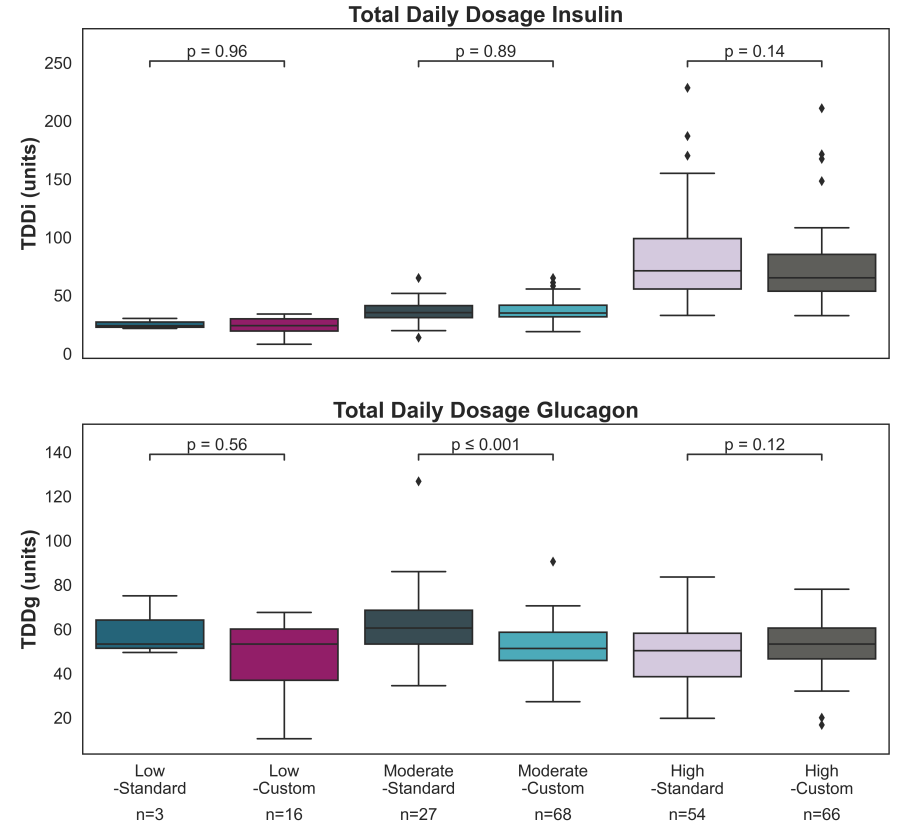


Figure 6.5: Hormone usage per ratio between glucagon setting and insulin curve setting. Standard is default glucagon settings. Custom is adapted to lower VI and/or EI glucagon setting. Low <25% insulin curve, Moderate 25-50% and High >50%. n = number of periods

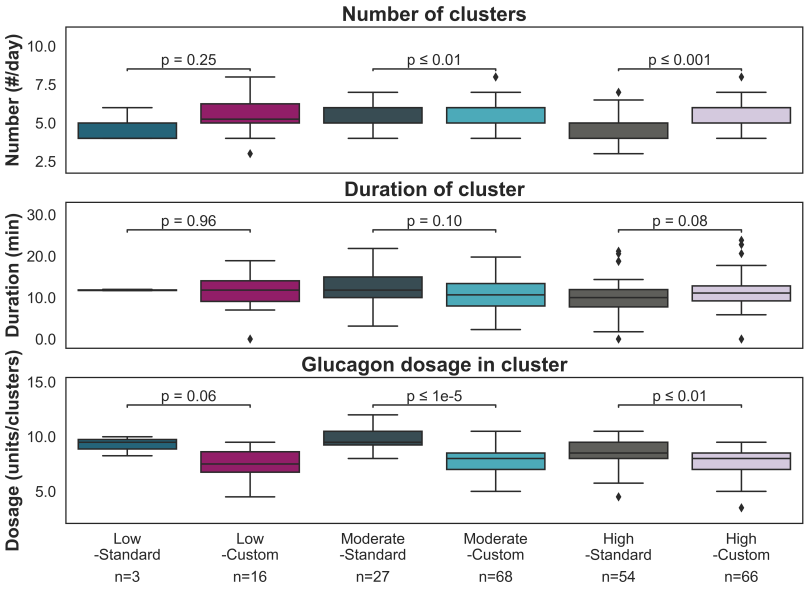


Figure 6.6: Cluster parameters per ratio between glucagon setting and insulin curve setting. Standard is default glucagon settings. Custom is adapted to lower VI and/or EI glucagon setting. Low <25% insulin curve, Moderate 25-50% and High >50%. n = number of periods

6.3.4 Change in glucagon setting

Comparing periods before and after change in glucagon setting reveals the following results: Firstly, changes in glucagon setting shows that all ranges could change in both directions but are within the variation seen in the reference group (see Appendix D.11). In general, the ranges do not change more than 10 minutes per day. Secondly, figure 6.7 shows the difference in TDDi and TDDg when glucagon settings are changed. Glucagon use is less when glucagon settings are reduced. Nevertheless, an increase in glucagon settings does not significantly change the glucagon usage. Moreover, insulin usage is not significantly affected by adaption of glucagon settings. Thirdly, figure 6.8 shows the outcome parameters at cluster level. Lowering glucagon settings in general caused a decrease in the amount of clusters per day, decrease in cluster duration and less units of glucagon in a cluster. Increasing the glucagon dosage exhibits a similar effect as decreasing, except that the glucagon dosage per cluster remains constant.

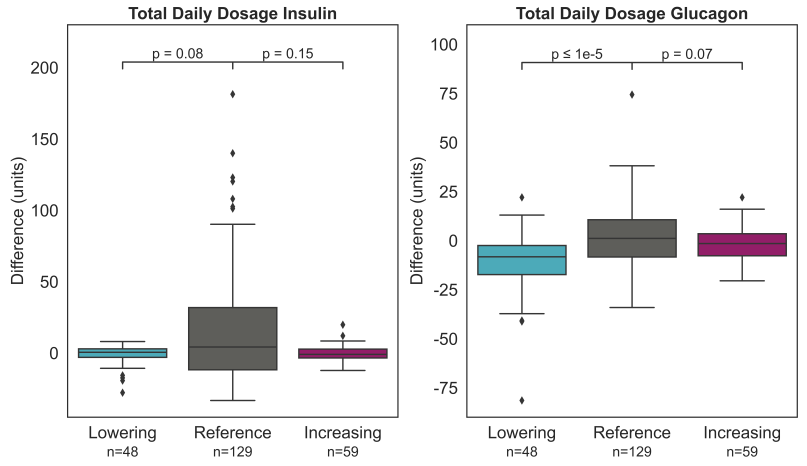


Figure 6.7: Hormone usage changes by modification glucagon settings. n is number of periods included.

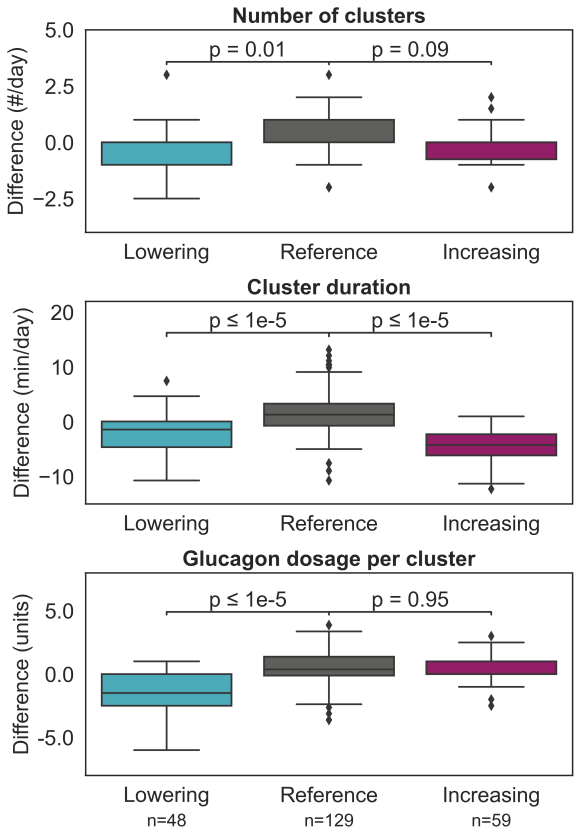


Figure 6.8: Cluster changes by modification glucagon settings. n is number of periods included.

6.4 Discussion

Summary of findings

The results shows that no specific glucagon settings give better glucose management or less hormone use. Hormone use remains constant across all glucagon settings, given that most clusters consist of a single VI. Yet, lower settings are associated with a higher number of clusters per day. Analysis adjusted for insulin sensitivity shows that for the high insulin curve setting only, TBR is slightly elevated by patients whereby the glucagon settings are customized. Moreover, insulin usage is more dependent on insulin curve settings than glucagon settings. On average, adapting glucagon settings does not affect the overall glucose management neither the insulin use. However, with lowered glucagon settings, the total glucose use is reduced as the clusters occurring less often and clusters contain less glucagon dosages without increase in cluster duration. This suggests that reducing glucagon settings is justified, as fewer amount of glucagon in a cluster is required and it does not take longer to slow down the glucose decline.

Absolute glucagon settings and ratio between settings

At the group level, no significant difference is found in overall treatment goals across various glucagon settings, suggesting that a one-size-fits-all approach is not applicable. However, differences in insulin use is found across various glucagon settings. Patients with higher glucagon VI and EI shows higher TDDi than patients with custom settings suggesting that TDDi is lower by custom glucagon settings. Nevertheless, these results may be biased by the fact that more patients with lower insulin curve settings also have reduced glucagon settings, given that insulin settings more strongly influence insulin use. It appears that patients with higher insulin sensitivity, as indicated by lower insulin curve settings, are more likely to obtain lower glucagon settings, probably caused by a higher glucagon sensitivity. In contrast, the patients with higher

insulin curve settings and lower glucagon settings, showed more time below range than patients with standard glucagon settings. A possible explanation could be that these patients have received more insulin, whereby glucagon has a reduced impact on the glucose levels resulting in more time below range. So, at group level insulin curve setting results as indicator to glucagon sensitivity. Therefore insulin curve settings could be taken into account by determine glucagon settings, where high insulin curve setting corresponds with high glucagon setting and low insulin curve with lower glucagon setting.

Change in glucagon setting

As the preceding paragraph addresses the group-level results, this section dives into the specifics of what can be anticipated when making individualized setting changes. When glucagon settings are changed, all three ranges can change both increasing and decreasing. However, on average is does not deviate much from the original ranges and the average is within variations seen in the control group. An important expected change is the potential decrease in glucagon use, attributed to a reduction in dosage per cluster and the number of clusters per day, without an increase in cluster duration. If the cluster duration increases, it may indicate that insufficient glucagon is being administered to effectively slow down the decline and maintain glucose levels within the desired range. Moreover, it is not yet clear if reduction of the number of clusters can be attributed to prevention of the jojo-effect which are caused by glucagon injections. Nevertheless, it is crucial to evaluate whether these adaptations yield the desired response on an individual basis as this varies between individuals.

Strength, limitations and further research

This study investigated the effect of glucagon settings on multiple levels (overall glucose management, hormone use and clusters) in real world data. As these levels give more insights in differences seen between different settings, this data has a disadvantage of selection bias. Patients who has customized settings, have shown indications of increased reaction to glucagon or experience side effects. Therefore, the results only apply to patients which shown indications to decrease settings. It does not provide information what customization does in the other patients without indications of decreasing settings. Nevertheless, this study presents general findings that could be expected, potentially easing the boundary or providing additional rationale to reduce glucagon settings for healthcare professionals. Another limitation of this study lies in the inclusion of periods. Patients with unmodified glucagon settings throughout the study period are represented by only one period, while other patients have more periods and are included multiple times, potentially overemphasizing a select group of patients. Nevertheless, a patient centered approach is needed in treatment of diabetes and glucagon settings should only be adapted to a selected group of patients which has indications such as jo-jo effect or side effects of higher glucagon dosages. This study shows evidence what changes in glucagon settings does in real world data at group level. Further research should validate quantitative measures which can be monitored in individuals to assess the effects of customization of glucagon settings, as this could reduce assessment time for healthcare providers and improve patient outcomes.

Conclusion

In conclusion, this study aims to investigate the impact of glucagon settings on the glucose regulation. Absolute glucagon settings did not show on average difference in overall glucose management en hormone usage. This indicates that there is no one-fits-all glucagon setting for all patients. Patients with high insulin sensitivity were more likely that glucagon settings were decreased. However, these patients uses on average the same amount of glucagon as patients with standard settings. Nevertheless, decreasing glucagon settings within a person could result in less glucagon use in that individual but with a reduced cluster duration and preserved TBR.

7 Patient Clustering for Optimized Glucagon Settings

7.1 Objective

The previous chapters showed the need of customization of glucagon settings in the bihormonal closed-loop system. In this chapter, cluster analysis will be performed to group patients based on the prediction of how well they respond to glucagon. This method might enables the customization of glucagon settings for specific patient clusters, promoting personalized approaches from the start of the treatment.

7.2 Methods

7.2.1 Dataset

The variables included in this study are numerical data which could be known before the start of treatment, originated from Chapter 5 and 6: BMI (kg/m^2), HbA1c (mmol/mol), age (years), diabetes duration (years), $TDDi_{bAP}$ (units).

7.2.2 PCA

Principal Component Analysis (PCA) is conducted to assess the explained variability of the variables, with the potential goal of reducing many correlated variables in the dataset. PCA is affected by scale, therefore the variables are scaled by subtracting the mean and dividing by the standard deviation. Thereafter, two PCA are executed using sklearn package. The first PCA only contains BMI and $TDDi_{bAP}$, as these variables shows correlation with glucose response to glucagon. The second PCA is applied with all variables. Loading values are noted and a scree plot is made for each PCA. If the eigen value of the first principal component is more than 70% of total sum of the eigen values, i.e. explain more than 70% of the variability, the loading values are reviewed on importance of variables. If a variable in the first principal component adds little to the explanation of variability, it is omitted from the cluster analysis.

7.2.3 Data analysis

Variables as input for the cluster analysis are determined on outcome from the PCA. K-means approach is used to cluster the patients groups, as this is an straightforward and easy to interrupt method which is a great starting point. K-means clustering is sensitive to scaling therefore the variables are scaled in the same manner as during the preprocessing of PCA. Two clusters are chosen, as the patients should be distinguish between standard and custom settings. To assess whether the clusters accurately represent glucagon response and corresponding settings, a comparison of the glucagon settings among the patients within each cluster is conducted. Glucagon settings is reviewed by the minimal glucagon VI setting in the study period, as this shows the most distinction between the desired setting. The outcome measures include sensitivity, indicating the percentage of patients with custom settings in the study period clustered in the custom setting group, and specificity, representing the percentage of patients with standard settings assigned standard settings.

7.3 Results

7.3.1 Dataset

Seventy patients with known $TDDi_{bAP}$ and BMI were included in the study. All variables were available for 66 patients. Custom settings (glucagon VI and/or EI) were utilized for 49 out of the 70 patients (70%). The numbers of patients with glucagon VI values of 11, 8, 6, and 5 units were 39 (56%), 22 (31%), 6 (9%), and 3 (4%), respectively.

7.3.2 PCA

Figure 7.1 shows the explained variance per component for PCA 1 and PCA 2. The explained variance represents the proportion of an eigenvalue for a component in relation to the total sum of eigenvalues. The first principal component of PCA 1 explains 75% of the observed variance. The corresponding loading values of component 1 are 0.707 for both variables BMI and TDDi, meaning that both contributing equal to the first principal component and are positively correlated. The second PCA did not show a component more than 35% of the sum of eigen values. Since no single variable accounts for a substantial portion of the variance, none of the variables are diminished for the cluster analyses.

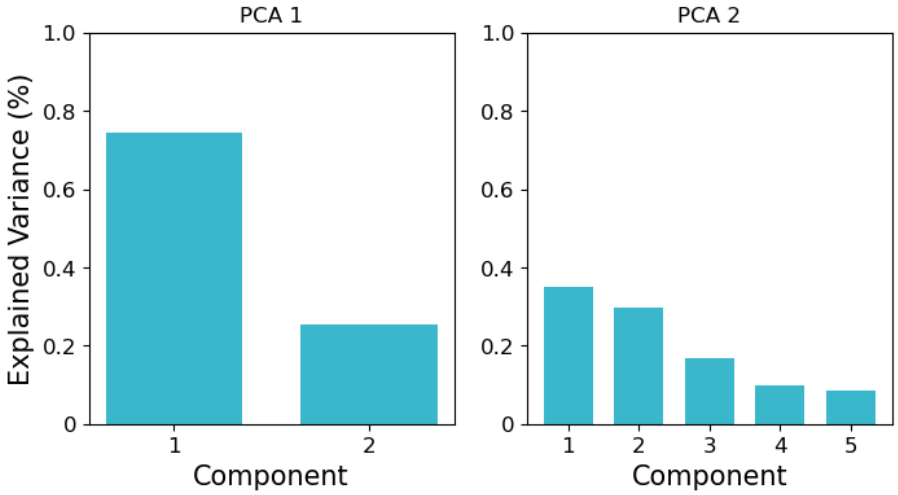
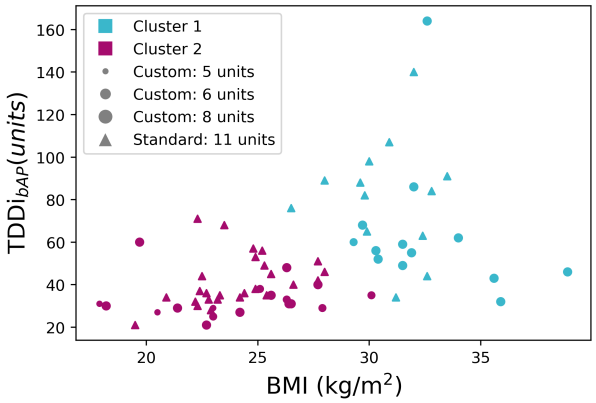


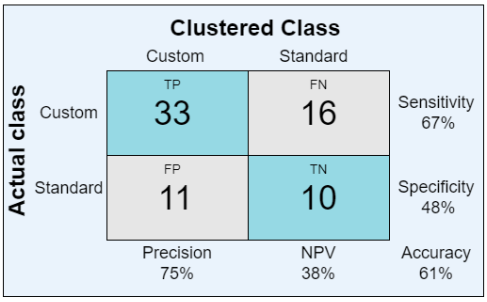
Figure 7.1: Scree plots. The variables used in the left plot consists of $TDDi_{bAP}$ and BMI. The right plot shows the results of the PCA with all variables included.

7.3.3 Cluster analysis

Figure 7.2 shows a scatter plot with assigned clusters and confusion matrix. What stands out is that the clusters are separated diagonally, indicating that both insulin usage and BMI are influential in defining the clusters. The scatterplot reveals that the clustering method with those two variables may not be effective, as the custom and standard settings do not exhibit clear separation based on the two variables. This suggests limitations in the ability of these variables, insulin usage, and BMI, to distinctly define the clusters. The sensitivity is higher than the specificity, indicating that this cluster model is better at predicting custom settings than standard settings. A precision of 75% has been achieved, indicating that 3 out of 4 patients who were predicted to belong to the custom cluster actually had custom settings during the treatment.



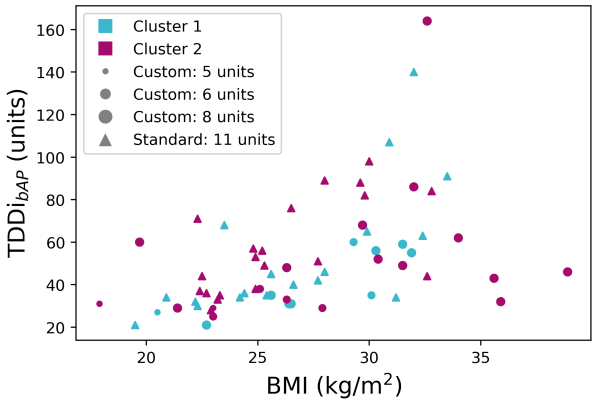
(a) Scatter plot displaying the relationship between BMI and Total Daily Insulin Use before AP treatment. Each marker represents a patient, where the color indicates the cluster assignment and the marker corresponds to standard or customized glucagon setting. The size of the marker corresponds to the minimal glucagon setting.



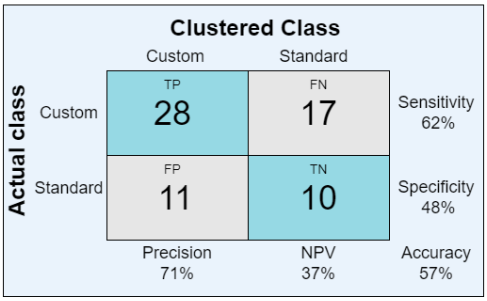
(b) Confusion matrix. TP = True Positive, TN = True Negative, FN = False Negative, FP = False positive. Sensitivity = TP/(TP+FN), specificity = TN/(TN + FP), Precision = TP/(TP+FP), NPV = Negative Predictive Value = TN/(TN+FN) and accuracy = (TP + TN)/(TP + TN + FP + FN)

Figure 7.2: Cluster algorithm applied to variables: BMI and TDDi$_{bAP}$

The results of the second cluster analysis are shown in Figure 7.3. The scatter plot shows the clustering assignment in the TDDi$_{bAP}$- BMI plot. It is notable that the cluster assignment appears random in this plane and does not accurately reflect the actual glucagon setting classes. As presented in the glucagon confusion matrix, the sensitivity is 62% and specificity 48%.



(a) Scatter plot displaying the relationship between BMI and Total Daily Insulin Use before AP treatment. Each marker represents a patient, where the color indicates the cluster assignment and the marker corresponds to standard or customized glucagon setting. The size of the marker corresponds to the minimal glucagon setting.



(b) Confusion matrix. TP = True Positive, TN = True Negative, FN = False Negative, FP = False positive. Sensitivity = TP/(TP+FN), specificity = TN/(TN + FP), Precision = TP/(TP+FP), NPV = Negative Predictive Value = TN/(TN+FN) and accuracy = (TP + TN)/(TP + TN + FP + FN)

Figure 7.3: Cluster algorithm applied to variables: BMI, TDDi$_{bAP}$, HbA1c, Age and Diabetes duration

7.4 Discussion

Summary

In summary, the observed variance cannot be adequately explained by a single or a couple of

factors. The PCA did not reveal a clear relationship or dominant factor influencing the variance observed in the dataset. The K-means cluster algorithm achieved a maximum sensitivity of 67% and a specificity of 48% to cluster patients with custom glucagon VI settings. The clustering performance did not improve with the inclusion of more values; instead, there was a slight decrease in sensitivity.

Application of clustering

Optimal settings at the start of AP treatment may offer benefits such as user-friendly initiation and reduced need for clinician interactions at the start. Therefore, the application of clustering in determining initial glucagon settings should meet certain performance criteria. Various considerations need to be taken into account in this regard. As glucagon functions as a hormone to counteract decreasing glucose levels and prevent hypoglycemia, ensuring an adequate administration is crucial. However, adjusting glucagon settings downward could result in potentially insufficient effects on glucose levels, as glucagon is dose-dependent. Therefore, it becomes necessary to carefully assess the possibility of decreasing glucagon settings while maintaining the desired impact on glucose regulation. On the other hand, minimizing the use of hormones is preferred, ensuring only essential amounts are administered. For instance, the intensification of insulin use may lead to weight gain and potentially increase insulin resistance [28]. The long-term effects of continuous glucagon dosages are not widely studied. Nonetheless, embracing a strategy of reduced hormone use not only holds the potential for improved health outcomes but also stands to benefit healthcare costs. Summing up, in a closed-loop system, the risk of underdosing glucagon outweighs the risks associated with overdosing. Hence, the clustering method in this study lacks the necessary high specificity for predicting custom glucagon settings.

Limitations

A limitation in this study is the comparison between clusters with minimal glucagon VI settings, as this may not always represent the optimal setting in all cases. In certain patients, there might be a potential to reduce glucagon settings. However, in current practice, adjustments to glucagon settings typically do not occur unless issues such as the jo-jo effect or excessive glucagon usage arise. In other patients, glucagon settings were adjusted in response to experienced side effects. However, these changes in settings may not always optimize the glucose regulation for these particular patients. Another limitation lies in the low ability to generalize the results to all AP users. The patient information prior to treatment was only accessible for a specific subset of patients, who, on average, exhibited a higher prevalence of custom glucagon settings compared to other patients included in the other studies within this thesis.

Conclusions en further research

The objective of this study was to assess if groups of patients can be identified which are comparable and benefit from the same glucagon settings. Although the performance of the clustering is not sufficient for direct implementation in practice, it has demonstrated that BMI and $TDDi_{bAP}$ maintain their value even when additional variables are excluded. Further research could explore other cluster algorithms as they have their own properties. Besides applying other cluster algorithms, the optimal glucagon settings for all patients should be determined. This could be by assessments of reports or by changing the glucagon settings till optimal settings. The initial method is safer for the patient and less time-consuming, but it is susceptible to interpretations by the researchers when compared to the experimental study design.

8 Advancing the use of glucagon in bihormonal closed-loop system

8.1 Introduction

With the introduction of the AP, fully automated controlling blood glucose levels is possible. One of the contributors to accomplish this, is the addition of glucagon dosages to the diabetes treatment. Little was known about continuous glucagon use in real-life setting. However, research is done with obtained data from real life use of the AP. This chapter provides education and offers advice for AP treatment for healthcare professionals.

8.2 Current practice

Glucagon settings will be adapted if the glucose curve shows indications which are qualitative reviewed by healthcare professionals. Indications could be occurrence of the jojo-effect, alternating pattern between glucagon and insulin injections accompanied by corresponding glucose level adjustments, caused by excessive response to glucagon (Figure 8.1a). Another indication could be that patients experience side effects like nausea by higher glucagon dosages. Glucagon settings will be reduced based on visual inspection of the glucagon dosages over multiple days, taking into account time of the day, presence of insulin VI or curve dosages. Type of setting is determined based on which injection VI or EI caused the most problems.

8.3 Scope of Recommendations

8.3.1 Healthcare professionals

Comprehending the Situation
It is important to identify the underlying causes of failures to maintain normoglyckemia, specifically focusing on the factors contributing to hypoglycemia in this context. First of all, the AP should not be paused manually or interrupted due to system errors such as glucagon blockage. Glucagon blockages ensures that no or too little glucagon is delivered into the subcutaneous tissue. Moreover, we known that insulin on board diminish the response to glucagon. So, if a lot of insulin is given before glucagon injections this could be the cause that glucagon could not prevent hypoglycemia. Therefore, insulin overdosing in bihormonal closed-loop is not effective [1]. Furthermore, regardless the amount of insulin on board, glucagon injections following rapid decreasing glucose levels also does not decline the decreasing glucose levels sufficient enough. Also, the blood glucose is an important factor as the effect of glucagon is less with higher blood glucose. In addition, it takes on average 10 to 20 minutes before glucose levels stops to drop and start to increase. If this takes longer, multiple times, this could be a sign of an absorption problem. It is noteworthy, that till 40 units of glucagon, the glucose levels is going to increase within 80 minutes after the first dosage.

Considerations and anticipated outcomes
The studies in this thesis indicated that females, patients with normal or low BMI, those with insulin curve settings less than 25%, and less <40 EH total daily insulin use, exhibited a faster and more elevated response to glucagon than their counterparts. So, these could be signs supporting a better fit with lower glucagon settings. Moreover, if patients are losing weight or

gaining a lot of weight, this could also be signs to reevaluate the insulin and also glucagon settings.

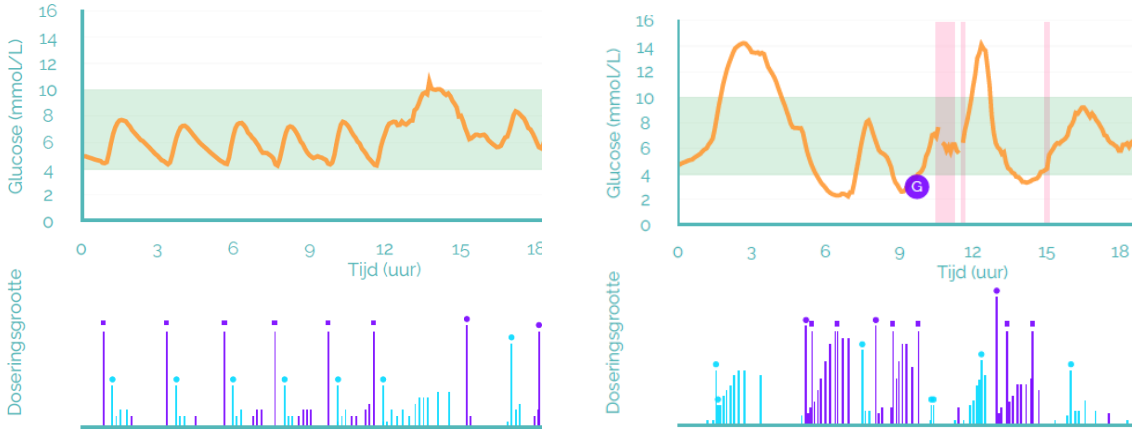
Data showed that, on average, when glucagon settings were lowered this does not affect the TBR, TIR and TAR, but it does reduce the glucagon use per day. Most of events which prevent hypoglycemia, are sufficient with a single VI injection. So, changing the VI has probably more effects on the regulation than EI. Reducing glucagon settings does not show less insulin usage and therefore reducing glucagon settings is not indicated to reduce insulin usage. It is advised to balance safe amount of glucagon but not give more than needed. A rule of thumb is that glucose levels in normoglyckemia do not necessarily need to increase, but they typically do not decrease significantly further into hypoglycemia for most instances when glucagon is dosed correctly. Thereby, it is important to evaluate when settings are changed if the desired effect is reached and not negatively affecting treatment goals.

Advice for medical support and healthcare professionals

To concretize the advice, consideration should be given to adjusted settings if glucagon does not prevent hypoglycemia, total daily glucagon usage is high (>60 units), give side-effects or responds excessive. A flowchart is provided in Appendix E.12 to assist healthcare providers in determining the appropriate steps to take. It is important to note that the flowchart should be used based on the general impression gathered over at least two weeks, rather than occasional occurrences. Firstly, consideration should be given to adjust insulin settings if, on multiple occasions and over several days, more than 2 units of insulin have been administered in the hour preceding glucagon dosage, or if the glucose level decreases by more than 6 mmol/L per hour at the time of glucagon dosage. The decision on which insulin setting to modify should be based on individual glucose patterns. For instance, if multiple dosages curve injections are given before glucagon dosages, then the insulin curve setting should be reduced. Moreover, if post-prandial peaks are already high (>12 mmol/L) and therefore it is not favorable to reduce the insulin VI setting, the insulin curve setting should be reduced. Secondly, in order to reduce the total daily glucagon dosages, overdosing of insulin should be excluded, after which the occurrence of the jojo effects can be examined. If the jojo-effect is caused or maintained by glucagon dosages, glucagon setting should be decreased depending on which injection is given, most likely VI. When the jojo-effect is not the cause of high glucagon usage, the glucagon EI should be reduced. Thirdly, if patients report side-effects from glucagon stacking. It could be considered to reduce the glucagon settings, preferably glucagon EI setting. However, the events where glucagon is not stacking should be reviewed to, especially when insulin curve setting > 50%, because the TBR might increase. If glucagon settings should be changed, it would be done in steps of 2 à 4 units till desired effect is reached or undesired effects appears. Fourthly, if patterns of excessive glucagon response are observed, it should be examined whether insufficient glucagon effect also occurs. In that case, no change should be made; otherwise, the glucagon setting, which is likely causing the jojo effect, should be reduced. Finally, it could be considered, when glucose regulation is stable, to determine glucagon settings based on TDDi and insulin curve settings, as lower usage and settings correspond to a more pronounced glucagon response. Those patients showed similar results with and without custom settings. This could indicates that in patients with standard settings, more glucagon is given than needed but it does not cause jojo-effect. However, it is advised to take into account the TBR and hypo-unawareness of the patient, to safely adapt the glucagon setting.

8.3.2 Developers

For developers of the AP system, there are a couple of aspects that warrant attention regarding glucagon implementation. Firstly, setting a limit for glucagon dosages within a specific time frame is desirable. Currently, glucagon is limited to the amount contained in the ampule thus a maximum of 100 units of glucagon. However, the results suggest that there are events where a lot of glucagon is administered with little to no effect (See Figure 8.1b). It is unknown what causes these events and if less glucagon gives the similar response. Nevertheless, some patients experience those events as annoying because they get nauseous and still have to eat. Possible causes could be a steep decreasing slope, infusion problems or absorption problems. In the first cases, it could be beneficial to warn patients earlier by steep decreasing slope with no effect of glucagon instead of giving an eat alarm at a glucose level of 3.5 mmol/L or lower. Additional alarms reduce the autonomic regulation of the AP and therefore, alarm criteria should be chosen with caution, so the alarm is not triggered too much but only when needed. Last of all, regardless of the already compensated dosages for glucose slopes, it still shows differences in response to glucagon, which could indicate that too few glucagon is given in those situations. These responses did not show a difference between insulin given in the past hour or not and are mainly dependent on the preceding glucose slope. So, the AP could be improved by setting a limitation to glucagon and set an additional criterion to the eat warning. However, the data analysis done in this thesis are not sufficient to determine the limits, therefore other comparisons should be made. However, this study suggest limitation around 50 units, as this did not show glucose from dropping within 80 minutes.



(a) Typical jojo-effect caused by glucagon

(b) Situations when glucagon limitation or eat alarm is desired

Figure 8.1: Examples from reports. The top graph shows the glucose curve and the bottom graph shows injections. Blue represent insulin and purple glucagon injections.

9 General discussion

Chapter 3 evaluates the impact of glucagon on glucose levels within a closed-loop system, examining responses with and without glucagon blockade in general. In Chapter 4, the influence of insulin on board on the glucagon response was explored. In Chapter 5, various inter- and intra-variables are assessed, which contribute to the effect size of the glucose response. Chapter 6 describes the effect of glucagon settings on the treatment with the Inreda AP. Chapter 7 applied and evaluate cluster analyses to predict glucagon settings before the start of treatment with the AP. Finally, Chapter 8 provides some considerations and advises regarding glucagon use in the AP. This Chapter aims to provide a critical examination of glucagon use in a bihormonal closed-loop and the limitations of this thesis, accompanied by recommendations for future research. Lastly, the relevance of this thesis will be discussed.

9.1 Real world data

The innovative aspect of this thesis is its exploration of the relatively unexplored effect of glucagon effect on glucose levels in real-life data. Researching the direct impact on glucose levels shed light on how glucagon enhance the successes and limitations of the bihormonal closed-loop system rather than simply confirming its effectiveness. Using real-world data ensures that factors which are typically overlooked or consciously disregarded in controlled studies are incorporated, providing a more realistic view on the role of glucagon in the bihormonal closed-loop system. Additionally, real world data opens the possibility of acquiring more data easily with a bihormonal closed-loop system than in controlled settings, as the system is used 24/7 by the patients. However, real world data could be complex to insulate one factor to investigate and assign results to this. Major factors affecting the results of glucagon response in this thesis are meals and exercises.

9.1.1 Meals

Meals increase blood glucose levels just like glucagon does. Therefore, meals possibly overestimate the glucose raising effects of glucagon. In the studies, this effects is as good as possible excluded to include analyses with night time data. However, night time data does also exclude other factors like insulin on board, stress and exercises.

Apart from the potential bias introduced by meals affecting the glucagon response, it would be advantageous to be able to isolate the effects of glucagon from meals during the assessment of reports. Healthcare professionals could better assess the glucagon sensitivity of that patient and consequently determine appropriate treatment options. As patients cannot announce their meals in the AP, another method should be applied to exclude clusters affected by meals. One method could be to exclude cluster with glucose excursion of more than 4 mmol/L, as this was the maximum glucose excursion in clinical setting to glucagon [4]. Nevertheless, this threshold measure is ambiguous as it could exclude strong glucagon responses. Another methods which could be applied is machine learning techniques to recognize patterns and thus classify as glucagon or meal intake. This method requires data to be classified to train and validate the model. A challenge could be that a lot of factors are involved in the glucose excursion after a meal for instance amount of carbohydrates intake, meal composition but also timing of insulin

and insulin sensitivity. The model should have a high sensitivity as the effect should mainly ascribe to glucagon. A well-trained model could support with assessment of glucagon response. Moreover, it might form the basis for a self-learning algorithm for glucagon.

9.1.2 Exercise

Besides meals, another factor affecting glucose regulation is exercise. Exercise is known to increase glucose uptake by muscles and increase insulin sensitivity which could be a challenge in reactive fully closed-loop system. As exercise affects the insulin sensitivity, insulin dosages before exercise could decrease glucose levels more than intended leading to hypoglycemia. A pilot study with Inreda AP have shown results that besides insulin dosages before exercise, glucagon was able to prevent post-exercise hypoglycemia in 7 out of 10 participants [33]. Bisgaard et al. described an endogenous glucagon release in T1DM during exercise, however this is attenuated compared to non-diabetic individuals [2]. It remains uncertain whether exercise influences glucagon sensitivity, potentially enabling the success of glucagon in a closed-loop system without the need for correction by exercise. The Inreda AP system have three accelerometers, on each sensor one and one on the computer/pomp itself. Unfortunately, this data is already filtered and processed into a measure which is saved to the database therefore a reliable measure for exercise is not yet available. Further studies could utilize IMA values (Integrated Modulus of body Acceleration) as measure of energy expenditure during physical activity, given its high accuracy [6]. The impact of accelerometer placement and orientation on the human body is negligible concerning the correlation. Besides that a reliable measure for activity could investigate glucagon sensitivity during exercise, it could also support data analysis as less assumption have to be made when patients are asleep or exercising.

9.1.3 Other confounding factors

Other confounding factors which could have influenced the results are software updates, errors in the sensor measurements and reliability of glucagon blockage detection. It's important to note that these are limitations inherent to the current Inreda AP system rather than limitations of the studies performed in this thesis itself. First, in the study period, a new software update has roll-out which has affect the control system and some reduced glucagon settings are increased to maintain safe use. The effect size to the results is unknown but the most impact would be visible in the investigation of glucagon settings, specifically the assessment of increase in glucagon settings. Second, glucose sensors could show a significant error both directions which affects the results [12]. Inreda AP uses two sensors and a measure of reliability of the sensors. However, sensor problems are present issue and therefore the results could significantly deviate from the true blood glucose value. This had impact on the accurately of the glucose levels but moreover, undesired or mostly unneeded glucagon could be administered and these can be included in the analysis. Clusters with insufficient sensor data were excluded but stricter criteria should be implemented to rule out clusters affected by sensor problems, to obtain accurate representation of the glucagon response. Criteria could be higher threshold value for reliability measure or excluding jumps of a certain range in measured glucose value when primary sensor is changed. Lastly, glucagon blockages could be detected by the AP. However, glucagon blockages could be detected late especially in low glucagon dosages. In all studies glucagon blockages which are detected later in the cluster are included, however it could be feasible that no glucagon is administered. So, the effect of glucagon could be diminished. Nevertheless, it is expected that the numbers of these clusters will not contrast significantly with the other clusters, as glucagon blockages occur far less frequently than clusters of glucagon dosages. In conclu-

sion, a lot of factors might effect the results but the effect size on the results is difficult to assess.

9.2 Strength and limitations

On top of all the above mentioned factors which could have influenced the results, the methods applied in this thesis have some limitations. Firstly, the patients included in this study are the first patients who were allowed to use the AP in their daily life and did not dropout during the study phase. This could lead to a selection bias, as these patients were highly motivated and some of them were poor responders to standard diabetic care. This together with withdrawal of unsatisfied participants could lead to a patient group that does not truly represent the average Type 1 diabetic patients and as a result, the findings of this study may not be generalizable to other Type 1 diabetic patients. However, motivation does not appear to be a notable issue, as the average Time in Range (TIR) remains consistent over a one-year period [32].

Secondly, this thesis uses clusters of glucagon dosages to assess the glucose raising effect of glucagon. However, this approach does exclude the fact that glucagon is dose dependent. Glucagon is administered until a desired glucose level is reached, allowing for multiple dosages of glucagon with time intervals in between. Administering multiple doses with time intervals complicates the quantification of the pharmacodynamic effects of glucagon. One method to account for the dose dependency of glucagon is to explore each scenario by dividing the clusters into different units of glucagon administered. However, the number of clusters limits these divisions, as too few clusters are incorporated to draw reliable results.

Lastly, the data is limited in this thesis to determine the role of glucagon in the Inreda AP at individual level. This study primarily focused on average outcome measures, yet the disadvantage of solely looking at averages is that it can conceal the variability and unique needs of patients. An individual-centered perspective is highly recommended in diabetes care because it acknowledges the unique needs, preferences, and circumstances of each patient. Cluster analysis might reveal groups of patients sharing similar characteristics that correspond to the effect size of glucagon, suggesting a potential requirement for either lower or higher glucagon settings. However, the sample size in this thesis limits the use of artificial intelligence to predict optimal (glucagon) settings. Nevertheless, the results of this study may help to determine parameters in a mathematical simulation model. If a simulation model of sufficient performance is built, it could enable simulation with various settings to predict expected outcomes. Consequently, personalized optimal settings could be determined. Nevertheless, developing mathematical models of glucose metabolism in T1DM patients is not yet feasible, as it is complex due to the many factors influencing the glucose levels [5]. Another approach of personalising the role of glucagon in individuals is to utilize the cluster method employed in the first three studies of this thesis. Visualizing the average glucagon response in an individual and comparing it to the responses of patients with similar profiles, then optimizing based on the settings of these other patients. An advantage of this method is that it shows average response to glucagon and this is more straightforward to assess the glucagon sensitivity and probably less subjective to healthcare professional to evaluate. A disadvantage of this method is that enough data should be available before an average response can be calculated. So, glucagon settings could not be personalized at start of the study.

9.3 Conclusion

In conclusion, this thesis has successfully achieved the objectives:

1. *Assess and quantify the impact of glucagon administration on glucose levels.*
   In general glucagon dosages ensures a 0.7 mmol/L less decrease and 11 minutes less time to raise the glucose level in patient with Type 1 Diabetes. Nighttime data shows equal glucose trends by glucagon dosages and blockages.

2. *Analyze the interaction between insulin on board and the effectiveness of glucagon.*
   Insulin on board does reduce the impact of glucagon on the glucose response. Glucagon dosages with insulin on board show a 13 minutes longer and -0.25 mmol/L more pronounced decrease while also exhibiting a 0.49 mmol/L less increase within an 80-minute timeframe. However, preceding slope seems to be more determined factor to the effect size of glucagon than the amount of insulin on board. Therefore, it would be more meaningful to incorporate the preceding slope in the algorithm to determine the appropriate amount of glucagon than considering the insulin dosage in the past.

3. *Evaluate potential factors, both within and between individuals, that play a significant role in influencing the response to glucagon.*
   Assessment of inter variables as slope and BGM did show an inverse correlation with glucagon response even as intra-variable factors BMI. Gender, total daily insulin and HbA1c and insulin curve setting showed only a significant difference between a single group and the other groups. Age and diabetes duration did not affect the glucagon response, and meaningful conclusions about glucagon dosage could not be drawn.

4. *Investigate what impact glucagon settings have on the glucose regulation.*
   No specific glucagon settings show better glucose management or less hormone use and thus there is no one fits best glucagon setting for all patients. Correcting for insulin sensitivity did only show that lowered glucagon setting with low insulin sensitivity showed a higher TBR than at standard glucagon setting. Decreasing glucagon settings within a person could result in less glucagon use in that individual but with a perserved TBR and reduced time in need for glucagon dosages.

5. *Apply and evaluate a cluster method for predicting optimal glucagon settings.*
   The K-means clustering applied to the dataset with a limited sample size and a poorly reliable outcome measure results in insufficient specificity and sensitivity, making it unsuitable for direct application in practice. Further research is needed to personalize settings without the need of thorough and time-consuming observations of reports. Further research should investigate how to classify or select people before treatment which should have advantage by custom glucagon settings.

6. *Propose guidelines to optimize the utilization of glucagon within the Inreda AP.*
   The main goal of this thesis was to determine the role of glucagon in the bihormonal closed-loop system of Inreda Diabetic used in daily life. The findings have significant implications for the understanding of the effect of glucagon in daily life conditions and patients and how settings are affecting the glucose regulation. Although these findings provide understanding and support for healthcare providers, this research has not provided definitive new guidelines for personalizing glucagon settings in the individual. However, the results do support the policy of current treatment. General rules of thumbs are defined with the

most important being to reduce glucagon settings when glucose regulation is stable and insulin usage is low, rather than when an excessive glucagon response is observed.

REFERENCES

[1] P. A. Bakhtiani, L. M. Zhao, J. El Youssef, J. R. Castle, and W. K. Ward. A review of artificial pancreas technologies with an emphasis on bi-hormonal therapy. *Diabetes, Obesity and Metabolism*, 15(12):1065–1070, 2013.

[2] M. Bisgaard Bengtsen and N. Møller. Mini-review: Glucagon responses in type 1 diabetes–a matter of complexity. *Physiological Reports*, 9(16):e15009, 2021.

[3] H. Blauw, A. J. Onvlee, M. Klaassen, A. C. van Bon, and J. H. DeVries. Fully Closed Loop Glucose Control With a Bihormonal Artificial Pancreas in Adults With Type 1 Diabetes: An Outpatient, Randomized, Crossover Trial. *Diabetes Care*, 44(3):836–838, 01 2021.

[4] H. Blauw, I. Wendl, J. DeVries, T. Heise, T. Jax, and P. consortium. Pharmacokinetics and pharmacodynamics of various glucagon dosages at different blood glucose levels. *Diabetes, Obesity and Metabolism*, 18(1):34–39, 2016.

[5] L. Boormans. *Extension of the Eindhoven Diabetes Education Simulator with a subcutaneous glucagon absorption and glucagon action model to simulate postprandial responses for type 1 diabetes mellitus patients on bihormonal closed loop systems [CONFIDENTIAL]*. PhD thesis, 2023.

[6] C. Bouten, A. Sauren, M. Verduin, and J. Janssen. Effects of placement and orientation of body-fixed accelerometers on the assessment of energy expenditure during walking. *Medical and biological engineering and computing*, 35(1):50–56, 1997.

[7] M.-K. Cho and M.-Y. Kim. What affects quality of life for people with type 1 diabetes?: a cross-sectional observational study. *International Journal of Environmental Research and Public Health*, 18(14):7623, 2021.

[8] P. E. Cryer. The barrier of hypoglycemia in diabetes. *Diabetes*, 57(12):3169, 2008.

[9] Diabeter. Type 1 diabetes. https://diabeter.nl/nl/over-diabetes/type1diabetes.

[10] DiabetesFonds. Ook diabetes type 1 groeit. https://www.diabetesfonds.nl/over-diabetes/diabetes-in-het-algemeen/diabetes-in-cijfers/ook-diabetes-type-1-groeit.

[11] T. Donner and S. Sarkar. Insulin–pharmacology, therapeutic regimens, and principles of intensive insulin therapy. 2015.

[12] J. El Youssef, J. Castle, and W. K. Ward. A review of closed-loop algorithms for glycemic control in the treatment of type 1 diabetes. *Algorithms*, 2(1):518–532, 2009.

[13] J. El Youssef, J. R. Castle, P. A. Bakhtiani, A. Haidar, D. L. Branigan, M. Breen, and W. K. Ward. Quantification of the Glycemic Response to Microdoses of Subcutaneous Glucagon at Varying Insulin Levels. *Diabetes Care*, 37(11):3054–3060, 10 2014.

[14] G. A. Gregory, T. I. Robinson, S. E. Linklater, F. Wang, S. Colagiuri, C. de Beaufort, K. C. Donaghue, D. J. Magliano, J. Maniam, T. J. Orchard, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *The Lancet Diabetes & Endocrinology*, 10(10):741–760, 2022.

[15] L. Hinshaw, A. Mallad, C. Dalla Man, R. Basu, C. Cobelli, R. E. Carter, Y. C. Kudva, and A. Basu. Glucagon sensitivity and clearance in type 1 diabetes: insights from in vivo and in silico experiments. *American Journal of Physiology-Endocrinology and Metabolism*, 309(5):E474–E486, 2015.

[16] M. Infante, D. A. Baidal, M. R. Rickels, A. Fabbri, J. S. Skyler, R. Alejandro, and C. Ricordi. Dual-hormone artificial pancreas for management of type 1 diabetes: Recent progress and future directions. *Artificial organs*, 45(9):968–986, 2021.

[17] J. Kesavadev, B. Saboo, M. B. Krishna, and G. Krishnan. Evolution of insulin delivery devices: from syringes, pens, and pumps to diy artificial pancreas. *Diabetes Therapy*, 11(6):1251–1269, 2020.

[18] D. Liu, U. Adamson, P.-E. Lins, and N. Clausen-Sjöbom. An analysis of the glucagon response to hypoglycaemia in patients with type 1 diabetes and in healthy subjects. *Diabetic medicine*, 10(3):246–254, 1993.

[19] A. Meglio, B. Molina, and R. Oram. Type 1 diabetes. lancet. *The Lancet*, 391(10138):2449–2462, 2018.

[20] S. L. Noble, E. Johnston, and B. Walton. Insulin lispro: a fast-acting insulin analog. *American Family Physician*, 57(2):279–286, 1998.

[21] W. H. Organization. Diabetes, Apr 2023. https://www.who.int/news-room/fact-sheets/detail/diabetes.

[22] J. Plank, A. Wutte, G. Brunner, A. Siebenhofer, B. Semlitsch, R. Sommer, S. Hirschberger, and T. R. Pieber. A Direct Comparison of Insulin Aspart and Insulin Lispro in Patients With Type 1 Diabetes . *Diabetes Care*, 25(11):2053–2057, 11 2002.

[23] A. Ranjan, S. Schmidt, S. Madsbad, J. J. Holst, and K. Nørgaard. Effects of subcutaneous, low-dose glucagon on insulin-induced mild hypoglycaemia in patients with insulin pump treated type 1 diabetes. *Diabetes, Obesity and Metabolism*, 18(4):410–418, 2016.

[24] N. Reddy, N. Verma, and K. Dungan. Monitoring technologies-continuous glucose monitoring, mobile technology, biomarkers of glycemic control. *Endotext [Internet]*, 2020.

[25] H. Reiband, S. Schmidt, A. Ranjan, J. Holst, S. Madsbad, and K. Nørgaard. Dual-hormone treatment with insulin and glucagon in patients with type 1 diabetes. *Diabetes/metabolism research and reviews*, 31(7):672–679, 2015.

[26] RIVM. Levensverwachting mensen met diabetes aanzienlijk lager, Oct 2021. https://www.rivm.nl/nieuws/levensverwachting-mensen-met-diabetes-aanzienlijk-lager.

[27] S. J. Russell, F. H. El-Khatib, D. M. Nathan, and E. R. Damiano. Efficacy determinants of subcutaneous microdose glucagon during closed-loop control. *Journal of Diabetes Science and Technology*, 4(6):1288–1304, 2010. PMID: 21129323.

[28] D. Russell-Jones and R. Khan. Insulin-associated weight gain in diabetes–causes, effects and coping strategies. *Diabetes, Obesity and Metabolism*, 9(6):799–812, 2007.

[29] S. I. Sherwani, H. A. Khan, A. Ekhzaimy, A. Masood, and M. K. Sakharkar. Significance of hba1c test in diagnosis and prognosis of diabetic patients. *Biomarker insights*, 11:BMI–S38440, 2016.

[30] A. Siafarikas, R. J. Johnston, M. K. Bulsara, P. O'Leary, T. W. Jones, and E. A. Davis. Early loss of the glucagon response to hypoglycemia in adolescents with type 1 diabetes. *Diabetes care*, 35(8):1757–1762, 2012.

[31] G. J. Taborsky Jr. The physiology of glucagon. *Journal of Diabetes Science and Technology*, 4(6):1338–1344, 2010.

[32] A. van Bon, H. Blauw, T. Jansen, G. Laverman, T. Urgert, J. Geessink-Mennink, A. Mulder, M. Out, R. G. Veldman, A. Onvlee, et al. Bihormonal fully closed-loop system for the treatment of type 1 diabetes: a real-world multicentre, prospective, single-arm trial in the netherlands. *The Lancet Digital Health*, 2024.

[33] A. C. Van Bon, L. D. Jonker, R. Koebrugge, R. Koops, J. B. Hoekstra, and J. H. DeVries. Feasibility of a bihormonal closed-loop system to control postexercise and postprandial glucose excursions. *Journal of diabetes science and technology*, 6(5):1114–1122, 2012.

[34] R. Ziegler, G. Freckmann, and L. Heinemann. Boluses in insulin therapy: a commentary. *Journal of Diabetes Science and Technology*, 11(1):165–171, 2017.

Appendix A

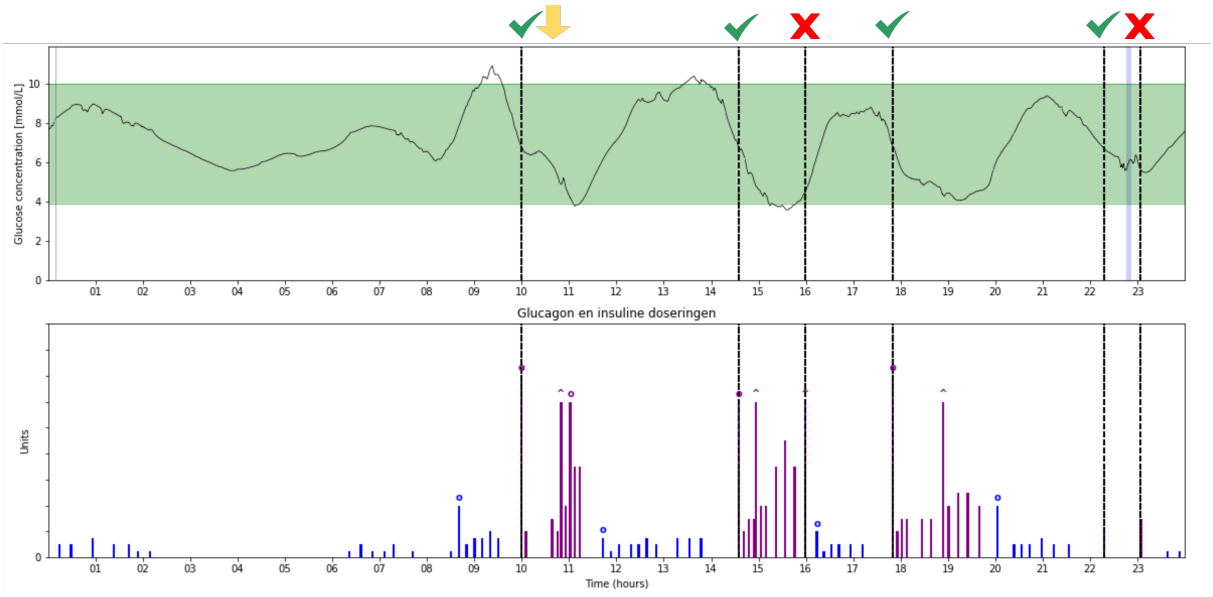


Figure 1: The graph displays the glucose curve (upper graph) and the corresponding insulin and glucagon dosages (lower graph) for a single day of one patient. The green area highlights the target range, while the blue area indicates periods when the AP is paused. In the lower graph, the blue lines represent insulin dosages, and the purple lines represent glucagon dosages. The height of the lines corresponds to the amount administered. Circles on top of the lines indicate dosage of type VI, while caret's (^) represent EI and without any symbol are dosages of type curve.

The vertical dashed lines are the start of a new cluster according to the cluster defining method. The marks on top of the upper graph represent the author's assessments. The first cluster is correctly assigned. Afterward, the glucose levels increases again, followed by a decrease with glucagon dosages. At this point, a new cluster should has been assigned. The second cluster is correctly assigned, but the third is not, as this dosage belong to the previous cluster. The fourth cluster is correct because the glucose curve decreases again, and the same applies for the fifth cluster. However, the last cluster is assigned incorrectly due to erratic sensor readings.

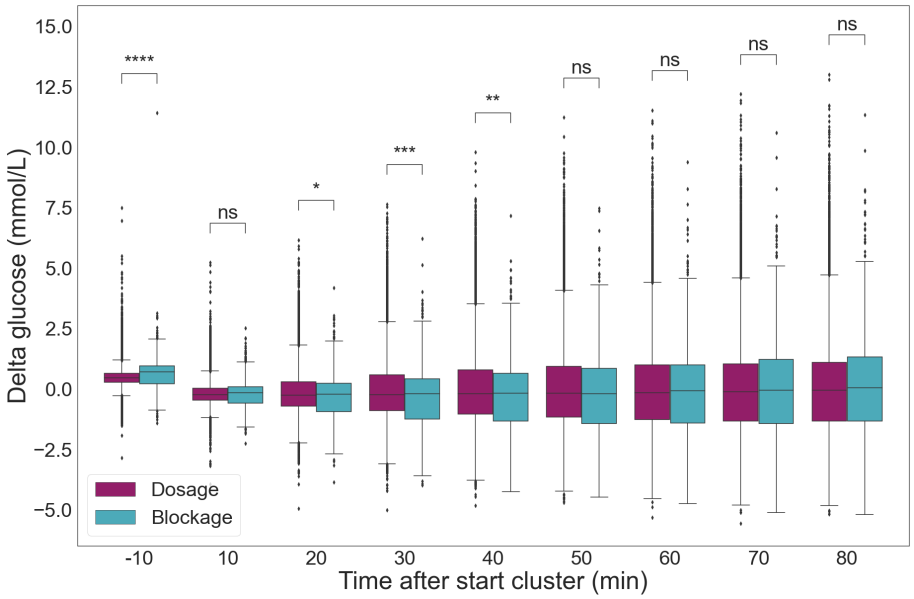


Figure 2: Boxplot analysis: glucagon response versus glucagon blockage during the night

Appendix B

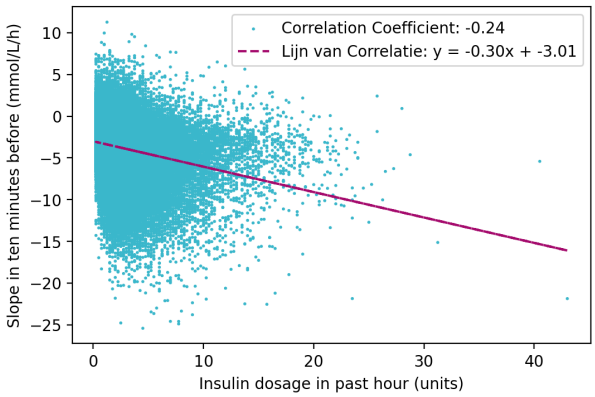
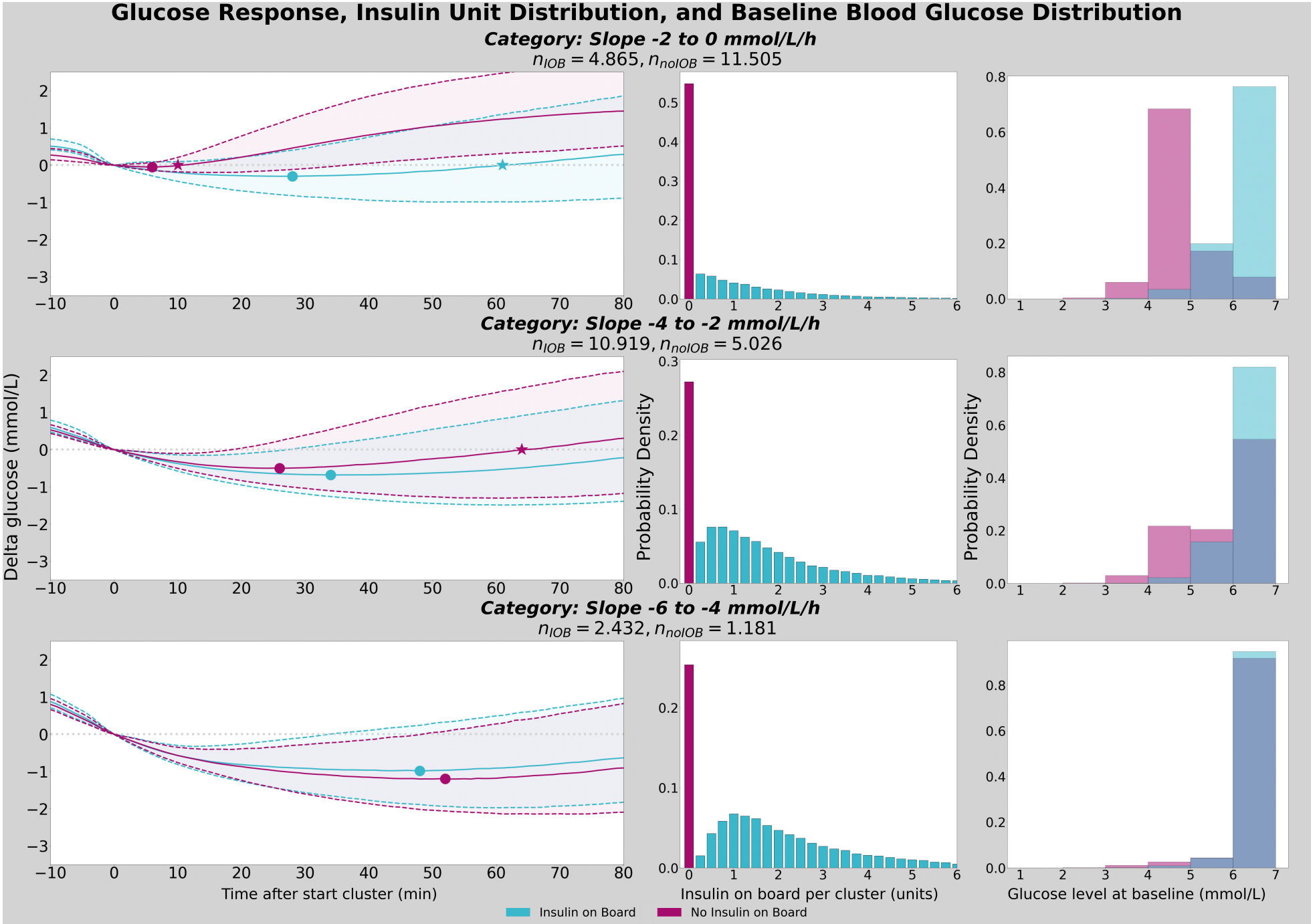


Figure 3: Correlation plot between preceding slope before start cluster and amount of insulin administered in past hour.


Glucose Response, Insulin Unit Distribution, and Baseline Blood Glucose Distribution
Category: Slope -2 to 0 mmol/L/h
$n_{IOB} = 4.865, n_{noIOB} = 11.505$
Category: Slope -4 to -2 mmol/L/h
$n_{IOB} = 10.919, n_{noIOB} = 5.026$
Category: Slope -6 to -4 mmol/L/h
$n_{IOB} = 2.432, n_{noIOB} = 1.181$
Delta glucose (mmol/L)
Probability Density
Time after start cluster (min)
Insulin on board per cluster (units)
Glucose level at baseline (mmol/L)
Insulin on Board
No Insulin on Board

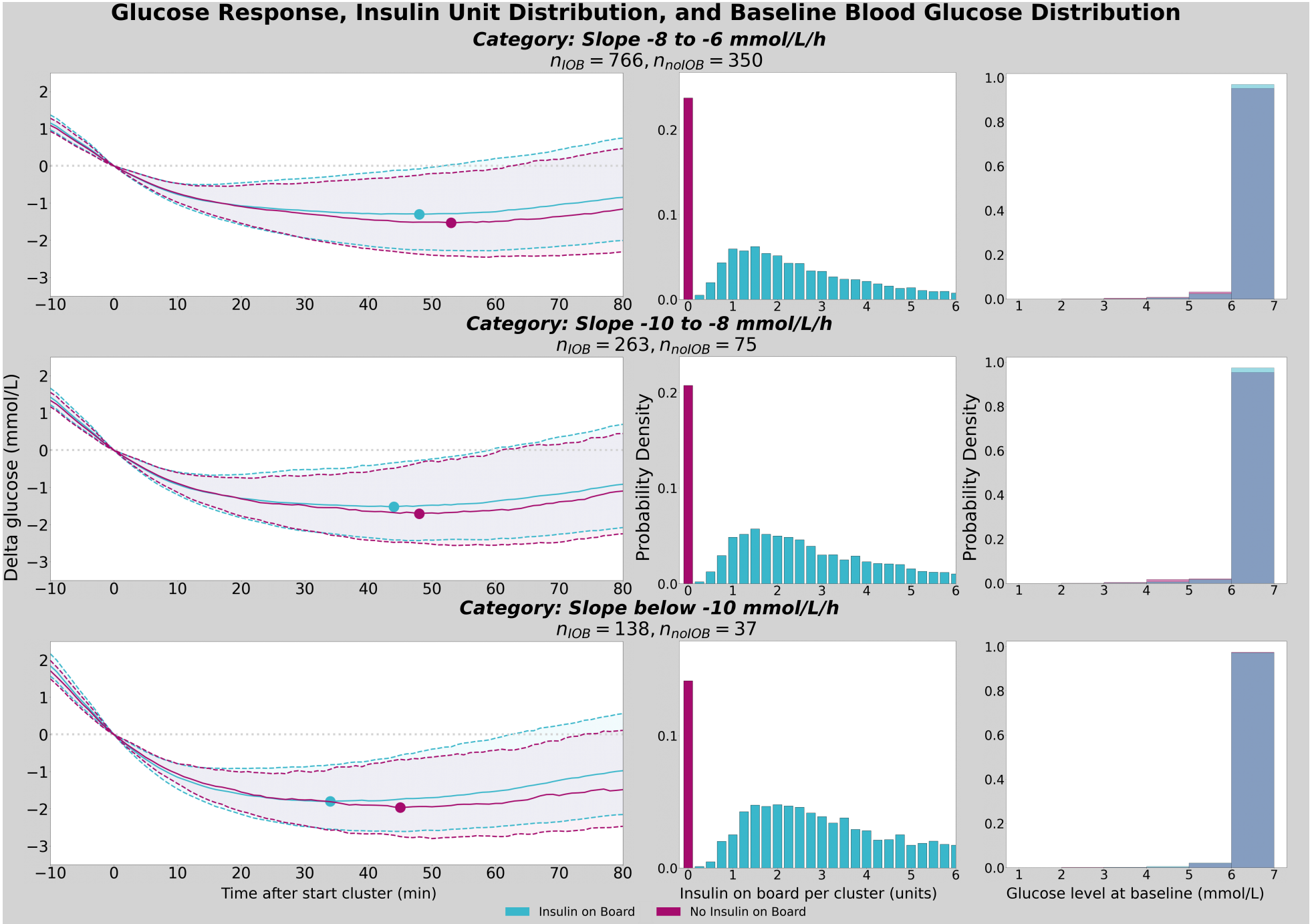

Glucose Response, Insulin Unit Distribution, and Baseline Blood Glucose Distribution
Category: Slope -8 to -6 mmol/L/h
$n_{IOB} = 766, n_{noIOB} = 350$
Category: Slope -10 to -8 mmol/L/h
$n_{IOB} = 263, n_{noIOB} = 75$
Category: Slope below -10 mmol/L/h
$n_{IOB} = 138, n_{noIOB} = 37$
Delta glucose (mmol/L)
Probability Density
Time after start cluster (min)
Insulin on board per cluster (units)
Glucose level at baseline (mmol/L)
Insulin on Board
No Insulin on Board

Figure 4: Slope analysis: Glucose Response, Insulin Unit Distribution, and Baseline Glucose Distribution during the night. The rows represent the categories; clusters with similar preceding slopes in order of faster descending slopes. The first column, shows the glucose response after glucagon dosage with (IOB, blue) and without (no IOB, red) insulin on board. The solid line represent the median reaction, the shaded area between the dashed lines is the range between the first and third interquartile. • is the point where the maximal decrease in glucose is reached and ★ were baseline glucose is recovered. The second column, shows the distribution of total amount insulin administered in the past hour before glucagon dosage, with the y-axis representing probability density. The last column shows the distribution of baseline glucose values measured at the first glucagon dosage for IOB and no IOB group.

Appendix C

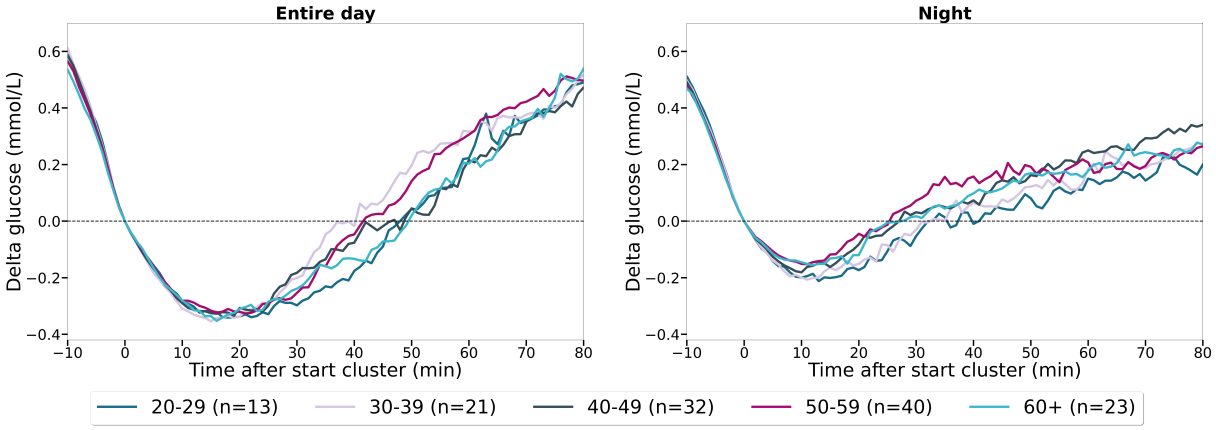


Figure 5: **Age**, median glucose response of group patients with different ages during the entire day and nighttime

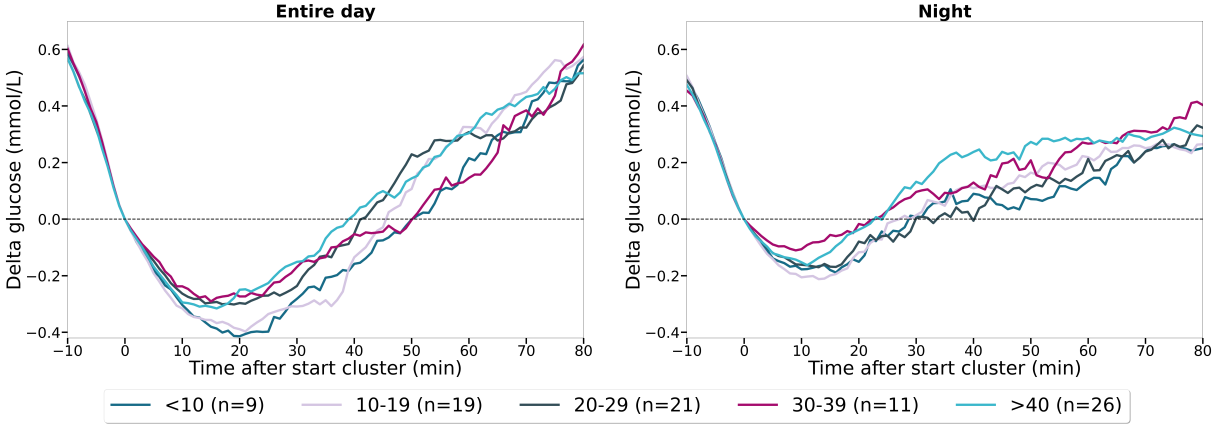


Figure 6: **Diabetes duration**, variability in median glucose response among Type 1 Diabetes patients with different durations of diagnosis.

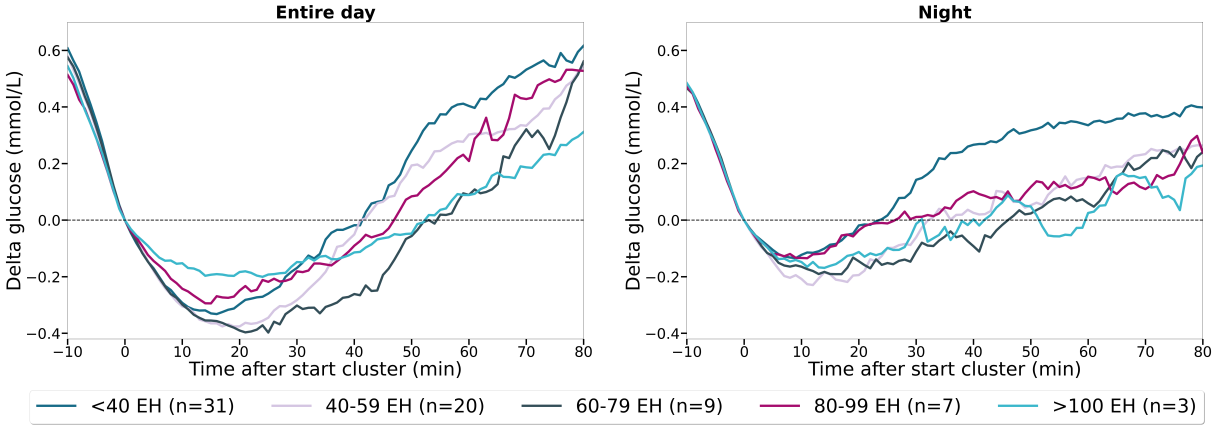


Figure 7: **TDDi$_{bAP}$**, variability in median glucose response among various total daily insulin dosage before AP treatment.

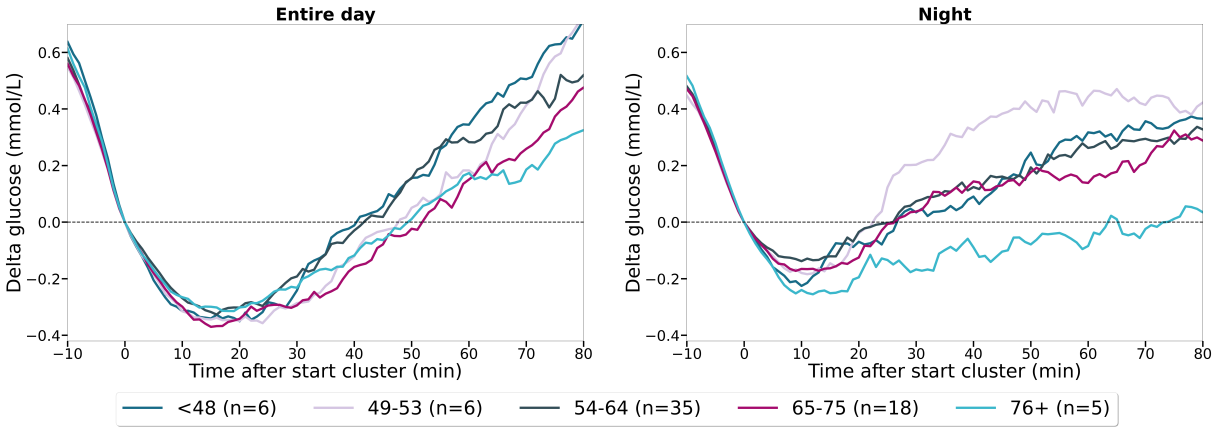


Figure 8: **HbA1c**, variability in median glucose response among patient groups with various HbA1c level before AP treatment.

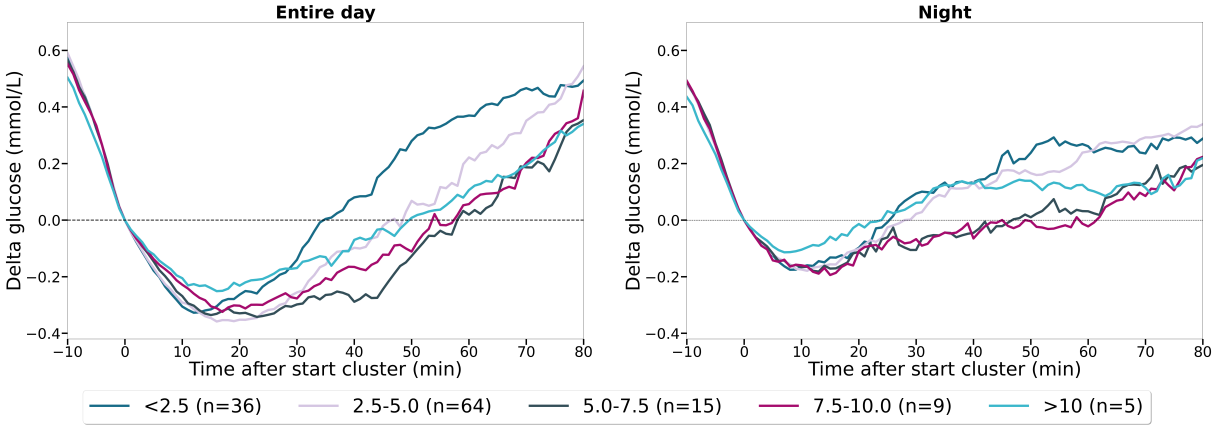


Figure 9: **I$_{VI}$**, variability in median glucose response among various insulin VI settings during the study

Appendix D

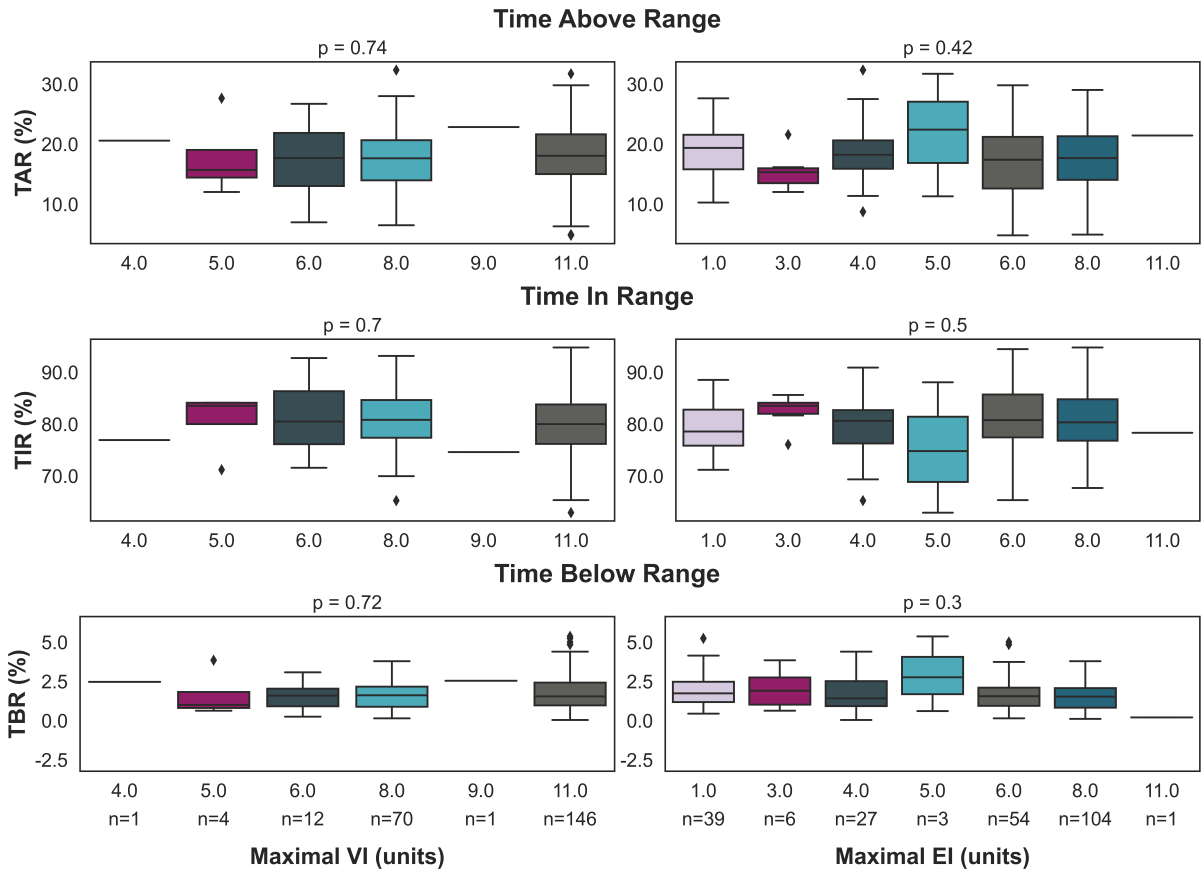


Figure 10: Overall glucose management per glucagon setting. N = number of periods.

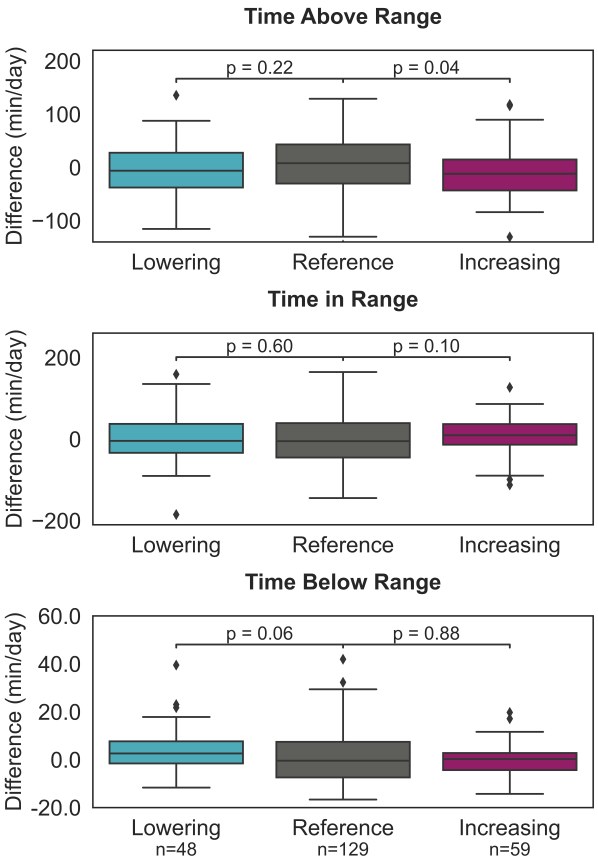
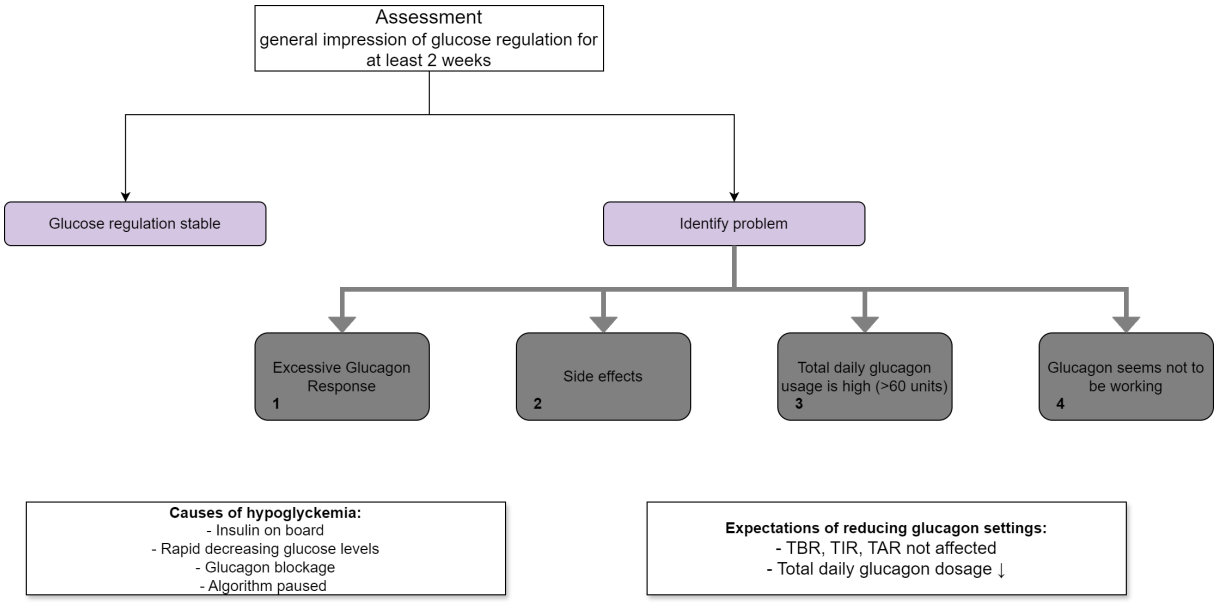
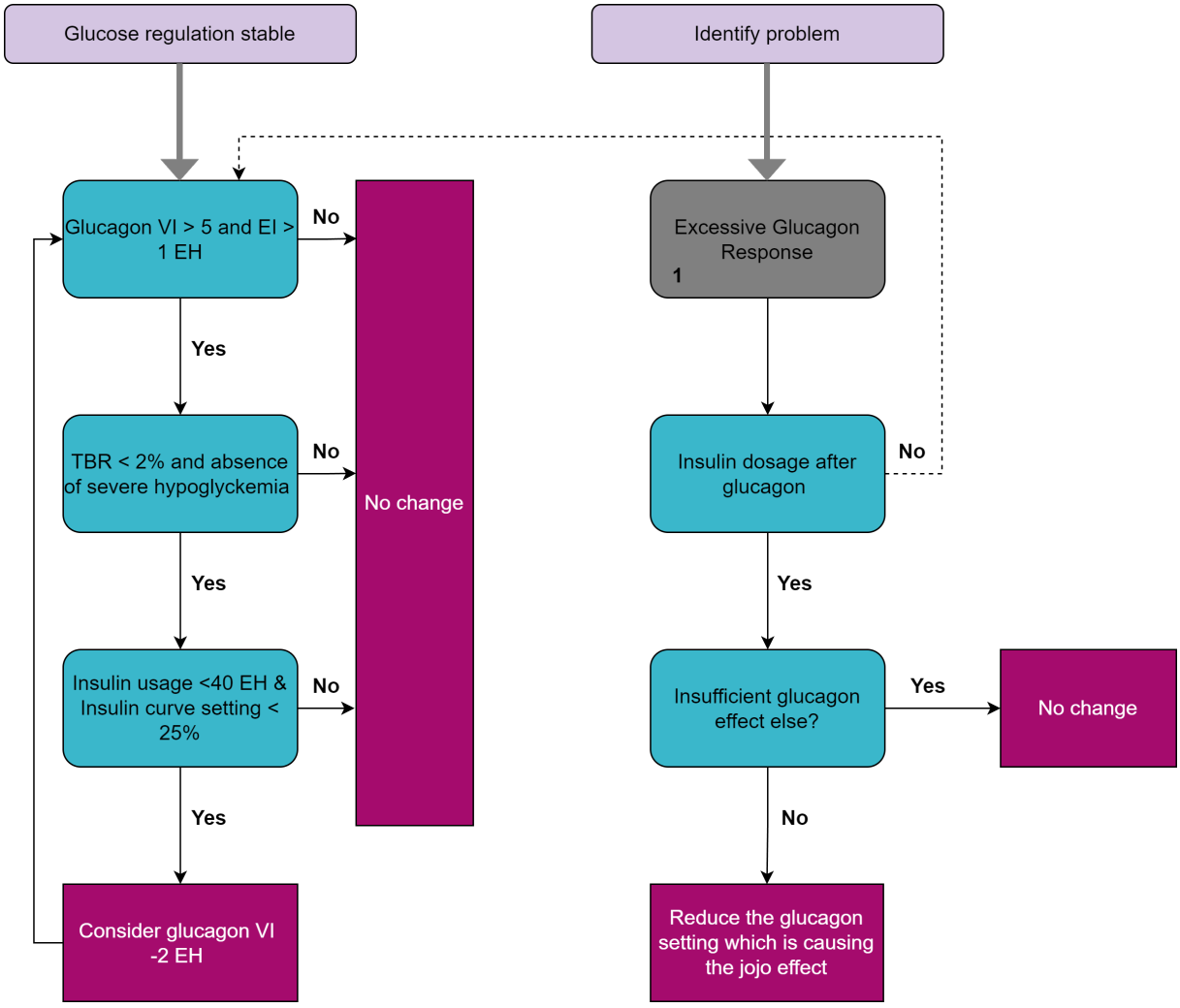


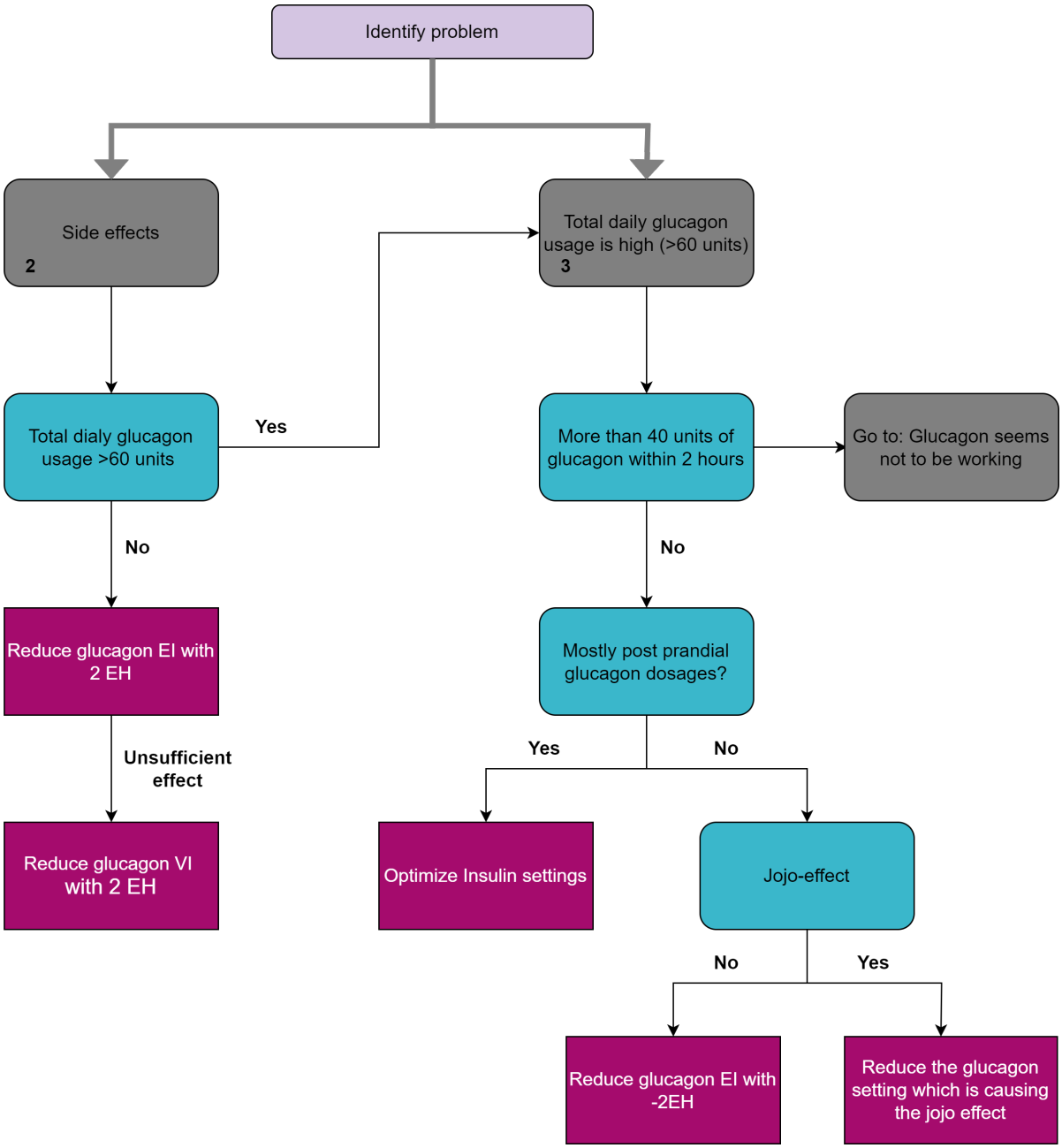
Figure 11: Overall glucose management changes by modification glucagon settings.

Appendix E

Figure 12: Flowchart of treatment plan for glucagon settings




Glucose regulation stable
Identify problem
Glucagon VI > 5 and EI > 1 EH
No
Yes
TBR < 2% and absence of severe hypoglyckemia
No
Yes
Insulin usage <40 EH & Insulin curve setting < 25%
No
Yes
No change
Consider glucagon VI -2 EH
Excessive Glucagon Response
1
Insulin dosage after glucagon
No
Yes
Insufficient glucagon effect else?
Yes
No change
No
Reduce the glucagon setting which is causing the jojo effect


Identify problem
Side effects
2
Total daily glucagon usage is high (>60 units)
3
Total dialy glucagon usage >60 units
Yes
More than 40 units of glucagon within 2 hours
Go to: Glucagon seems not to be working
No
No
Reduce glucagon EI with 2 EH
Mostly post prandial glucagon dosages?
Unsufficient effect
Yes
No
Reduce glucagon VI with 2 EH
Optimize Insulin settings
Jojo-effect
No
Yes
Reduce glucagon EI with -2EH
Reduce the glucagon setting which is causing the jojo effect

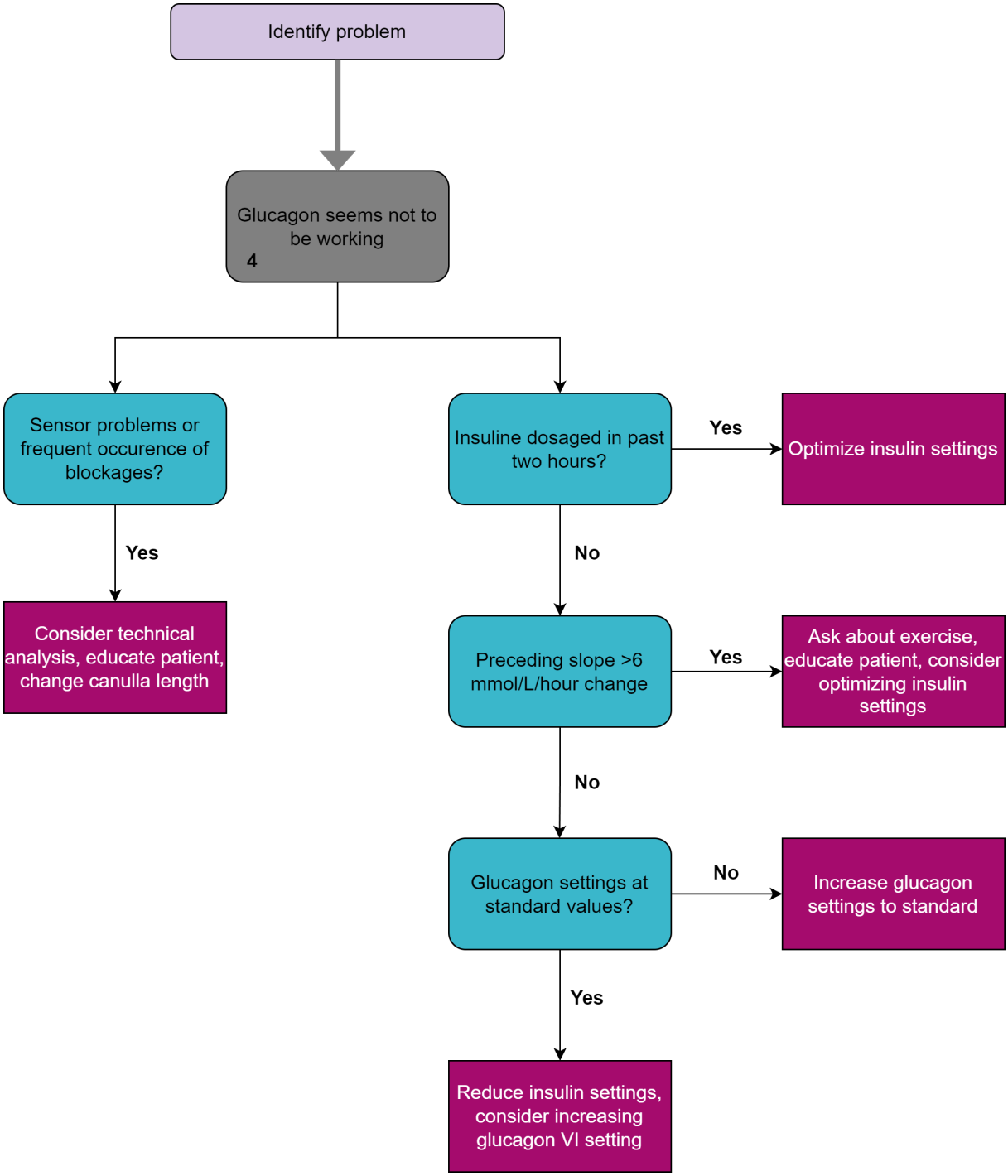

Identify problem
Glucagon seems not to be working
4
Sensor problems or frequent occurence of blockages?
Yes
Consider technical analysis, educate patient, change canulla length
Insuline dosaged in past two hours?
Yes
Optimize insulin settings
No
Preceding slope >6 mmol/L/hour change
Yes
Ask about exercise, educate patient, consider optimizing insulin settings
No
Glucagon settings at standard values?
No
Increase glucagon settings to standard
Yes
Reduce insulin settings, consider increasing glucagon VI setting

Figure 13: Proposal to determine start glucagon settings, which first needs validation in a study context

