Title page

Is low-dose glucagon needed and effective in preventing fasted exercise-induced hypoglycaemia in type 1 diabetes treated with the MiniMed 780G, an automated insulin delivery system?

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Clinical Trials Register: NCT05379686

Keywords: Hybrid closed loop, artificial pancreas, hypoglycaemia rescue and physical activity

Journal: Diabetes, Obesity and Metabolism

Abstract: 242 words

Main body of text: 2670

References: 27

Figures/tables: 3/2

Abstract

Aim:

To evaluate and compare the plasma glucose (PG) response during spontaneous fasted morning moderate-intensity exercise with and without injection of subcutaneous glucagon in adults with type 1 diabetes (T1D) treated with an automated insulin delivery (AID) system.

Methods: Ten adults (4 female) with T1D (age: 50 [42-67] years, diabetes duration: 22 [14-44] years, HbA1c: 55 [47-69] mmol/mol) treated with the MiniMed™ 780G AID system participated in a proof-of-concept two-period, crossover trial. Fasting participants undertook a 45 min bout of continuous moderate-intensity (~60% $\dot{V}O_{2peak}$) exercise on a cycle ergometer followed by 1 hour of rest. Before exercise, 150-microgram glucagon was administered subcutaneously on visit 1 (GLUC) but not on visit 2 (NO-GLUC). Temporary target on the AID was activated 15 minutes before until 15 minutes after exercise cessation. Blood samples were taken at 5- and 15-minute intervals for measuring PG and biomarkers. Data were analysed using paired t-tests or repeated measures ANOVA.

Results: Time in range (3.9–10.0 mmol/l) was 100% on both study visits. No hypoglycaemia (<3.9 mmol/l) occurred in either arm. The GLUC arm had significantly higher mean PG ($p=0.01$), area under the PG curve ($p=0.01$), coefficient of variation ($p<0.01$), peak PG ($p=0.01$), and PG at the end of exercise ($p<0.01$). No differences in endogenous glucoregulatory hormones were observed between visits.

Conclusion: Adults with T1D treated with the MiniMed™ 780G can perform spontaneous fasted moderate-intensity exercise without hypoglycaemia. Therefore, glucagon was not needed for prevention of hypoglycaemia in such situations.

1. INTRODUCTION

Automated insulin delivery (AID) systems have demonstrated significant improvements in achieving glycaemic targets by continuously predicting insulin needs, adjusting insulin delivery based on sensor glucose levels, and helping protect users from low glucose levels.[1,2] However, individuals with T1D treated with AID systems still need to plan for exercise by notifying the algorithm in advance, minimising insulin on board before starting exercise, and maybe also consuming additional carbohydrates around exercise to prevent hypoglycaemia.[3–5] Given the recent availability of the MiniMed™ 780G system there are currently only a limited number of research studies evaluating glucose control with this system under exercise conditions. These studies explore how to best optimise glycaemia around physical exercise through various carbohydrate strategies, different time settings for temporary glucose targets, and different insulin formulations in people with T1D treated with this AID system.[6–9] However, strategies that allow for greater spontaneity in exercise without the need for extensive pre-exercise carbohydrate adjustments and elevating the glucose target 1-2 hours before could be highly valuable. In recent years, a number of studies have successfully demonstrated that subcutaneous (s.c.) low-dose glucagon can be utilised to effectively prevent and treat exercise-induced mild hypoglycaemia in people with T1D.[10–12] Likewise, s.c. infusion of low-dose glucagon in dual-hormone closed loop systems has shown promising results in managing glucose levels.[13] Additionally, research indicates that the risk of exercise-induced hypoglycaemia when treated with non-AID systems is lower when exercising in the morning while fasted compared to exercise that is undertaken in the afternoon or evening.[14,15]

Therefore, this study aimed to evaluate glucose metrics to fasted morning moderate intensity exercise with and without subcutaneous glucagon in adults with T1D using an AID system.

2. METHODS AND MATERIALS

2.1 Study design and ethical approval

This was a single-center, cross-over, non-randomised, two-period study involving 10 participants with T1D treated with an AID system. The participants went through a screening visit and two experimental study

visits that were completed in sequential order. The study was conducted at the clinical research unit at Steno Diabetes Center Copenhagen, Herlev, Denmark. It was monitored by the Good Clinical Practice Unit at Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark, and approved by the Danish Medicines Agency (EudraCT 2021-004993-68), the Regional Committee on Health Research Ethics (H-21063661) and the Danish Data Protection Agency (P-2022-102). The study was registered at clinicaltrials.gov (NCT05379686) and conducted in accordance with the Declaration of Helsinki.

2.2 Participants

Participants were recruited from the outpatient diabetes clinic between March 2022 and May 2023. Key inclusion criteria were age ≥18 years, duration of T1D ≥2 years, being treated with the AID system MiniMed™ 780G (Medtronic, Northridge, CA, USA) ≥ 4 weeks and using insulin aspart (Novorapid, Novo Nordisk, Bagsværd, Denmark) ≥1 weeks. Key exclusion criteria were use of anti-diabetic medicine other than insulin, known allergy to glucagon or related products, females who were pregnant, or without contraception, and concomitant medical or psychological conditions considered to render the individual unsuitable for study participation.

2.3 Screening procedures

After having provided written and oral informed consent, participants completed the screening visit to evaluate eligibility. The visit included registration of baseline characteristics, urine and blood sample, a 12-lead electrocardiogram, a medical examination and a review of medical history and medications. A cardiopulmonary exercise test (CPET) was then performed to volitional exhaustion to obtain peak cardio-respiratory rates that were necessary to set sub-maximal cycle intensity on a workload-controlled cycle ergometer for the two main trials.[16]

2.4 Pre-study procedures

Participants were instructed to refrain from ethanol consumption, strenuous physical exercise, and try to avoid hypoglycaemia (sensor glucose or finger prick value <3.9 mmol/l) 24 hours before each study visit. Administration of glucose tablets prior to study initiation was allowed to avoid hypoglycaemia. Participants were also instructed not to administer bolus insulin 4 hours prior to the start of each study visit. To minimise

the potential for hypoglycaemia, all participants were advised to set a low alarm (4.5 mmol/l) on their pump. In instances where there was a low alarm, the participants were asked to verify it by a finger prick test. Continuous glucose monitoring (CGM) data for the 24 hours prior to each experimental visit were also evaluated on each study day to check for episodes of hypoglycaemia. If severe hypoglycaemia (<3.0 mmol/l) had occurred, the study visit was postponed for at least one day**.**

2.5 Experimental trial day procedures

On experimental trial visits, participants arrived at the research facility in the morning after an overnight fast. An intravenous catheter was inserted into an antecubital vein for venous blood sampling throughout the visit. For female participants of childbearing potential, a urine HCG test was performed to ensure a non-pregnant status.

After an initial 45-minute rested period, participants started exercising on a cycle ergometer at a fixed moderate intensity informed by the $\dot{V}O_{2peak}$ obtained at the screening visit (60 % of $\dot{V}O_{2peak}$) for 45 minutes. From 15 minutes before to 15 minutes after exercise (t=-15 to t=60 min), the temporary target in the AID system was activated. This function increases the target glucose level to 8.3 mmol/l and deactivates the autocorrection feature. Immediately before exercise start (t = 0 min) 150 µg s.c. glucagon was administrated in the abdominal area on visit 1 (GLUC). Native glucagon powder (GlucaGen®, Novo Nordisk, Bagsværd, Denmark) was used and was dissolved 10 minutes before administration. No glucagon was given on visit 2 (NO-GLUC). Each visit was separated by a wash-out period of ≥3 days.

Plasma glucose (PG) and lactate were immediately measured on a YSI 2900 STAT (Yellow Springs, Brannum Ln, OH, USA) every 5 minutes during exercise and every 15 minutes in the pre-exercise and 1-hour post-exercise observation periods. The remaining volume was stored at -80ºC after centrifugation and subsequently used to determine the concentration of insulin (Iso-Insulin Elisa, 10-1128-01, Mercodia, Uppsala, Sweden), glucagon[17], cortisol (Elecsys Cortisol II assay, CORT2, Application Code 10042, Cobas 402 and 801, Roche Diagnostics, Switzerland), growth hormone (Elecsys hGH assay, HGH, Application Code 10096, Cobas 402 and 801, Roche Diagnostics, Switzerland, adrenaline, and noradrenaline (sandwich adrenaline and noradrenaline, EA613/192, Eagle biosciences, Amherst, NH, USA) at 5-6 timepoints at

baseline, during and after exercise.

If hypoglycaemia (defined as PG < 3.9 mmol/L) had occurred during exercise, the activity would have been stopped, and 15 g of oral glucose would have been administered. The participant would then rest for the remainder of the intended 45-minute exercise session. If the PG did not rise to >3.9 mmol/L after 15 minutes, an additional 15 g of oral glucose would have been provided. Adverse events (nausea, stomach-ache, injections site pain, headache, and palpitation) were assessed using a 0-100 visual analogue score (VAS) before (baseline), after exercise, and at the end of the trial to evaluate the occurrence of any adverse events. The participants consumed a standardised low-glycaemic index, carbohydrate-based meal (0.75 g carbohydrates per kg body weight) with their usual meal-time insulin dose after the exercise and observational period before leaving the laboratory. Figure 1 illustrates the study visit.

2.6 End points

The primary endpoint was the difference in the percentage of time spent with PG concentrations within the target range (TIR [PG: 3.9 – 10.0 mmol/l]) during and for 1-hour after dynamic physical exercise (t=0 min to +105 min) between the two visits. The secondary endpoints were the differences seen between the two visits in the incidence rate of hypoglycaemic events (PG<3.9 mmol/l), difference in time (min) to hypoglycaemia (PG<3.9 mmol/l), difference in percentage of time below target (TBR [PG <3.9 mmol/l]) glucose range, difference in percentage of time above target (TAR [PG>10.0 mmol/l]) glucose range, difference in incidence rate of hyperglycaemia (PG>10.0 mmol/l), difference in nadir PG concentration, difference in peak PG concentration, difference in incremental peak PG concentration, difference in mean PG concentration, difference in PG Area Under the Curve (AUC), difference in standard deviation in PG concentrations and the difference in coefficient of variation in PG concentrations. All endpoints were from exercise initiation to end of the observation period.

2.7 Statistical analysis

This was a proof-of-concept study to assess the use of low-dose glucagon in AID system treated individuals with T1D performing exercise. The sample size of 10 participants was chosen from a feasibility perspective. Baseline characteristics are summarised using median with range of minimum and maximum values.

Assessments of continuous outcomes are presented as means with standard deviation or medians with interquartile ranges. Outcomes were compared between visits using a paired t-test for normally distributed data or the nonparametric equivalent Wilcoxon Signed Rank test. Data obtained from repeated measurements were analysed using a repeated measurement ANOVA followed by Tukey adjustment for multiple comparison. Missing PG values were extrapolated from the closest measurements using linear interpolation. Statistical analyses were performed with R studio 4.3.0 (R foundation for statistical computing, Vienna, Austria) and SAS 9.4 (SAS institute, Inc., Cary, NC, USA). P values of <0.05 were considered statistically significant.

3. RESULTS

3.1 Baseline characteristics

A total of 10 participants (4 females) were enrolled in, and completed, the trial. The baseline characteristics of the participants are presented in Table 1.

3.2 Plasma glucose responses

The glycaemic metrics for the combined exercise and post-exercise periods are shown in Table 2. TIR was 100% in both conditions (p=0.18). Time in tight range (TITR [PG: 3.9-7.8 mmol/l]) was 44% for the GLUC arm and 97% for the NO-GLUC arm (p=0.10). TBR and TAR were similar between visits (p>0.99, p=0.37), with no occurrences of hypoglycaemia or hyperglycaemia in either arm. The nadir PG concentration was comparable between visits (p=0.41). Peak PG concentration was 1.8 mmol/l higher in the GLUC arm compared to the NO-GLUC arm (p=0.01). The same trend was observed for delta PG and mean PG levels across visits (p=0.02, p=0.01). The area under the curve (AUC) for the entire trial day was 824.2 mmol/l*min for the GLUC visit and 686.2 mmol/l*min for the NO-GLUC visit (p=0.01). Incremental AUC was also higher in the GLUC arm (p<0.01). Standard deviation and coefficient of variation differed significantly between visits favouring the GLUC-arms (p=0.01, p<0.01). No significant differences were observed in PG levels at the start of exercise or at the end of the trial day between the two arms (p=0.54, p=0.07), but PG at the end of exercise was higher in the GLUC arm (p<0.01). Only one participant in the

GLUC-arm required a glucose tablet prior to the trial. This participant´s glucose peak was 8.12 mmol/l, closely aligned with the overall mean peak. No severe adverse events were reported in either condition.

3.3 Metabolic and hormonal biomarkers

Counterregulatory hormone levels were similar at baseline and throughout the trial (including baseline, peak, AUC, incremental AUC, and incremental peak measures; all p-values > 0.05), apart from glucagon due to the study design. Glucagon levels were significantly higher in the GLUC arm compared to the NO-GLUC arm at all time points (AUC: $p < 0.0001$, peak: $p=0.0001$, incremental AUC: $p=<0.0001$, incremental peak: $p=0.0002$)) except baseline ($p = 0.09$). Insulin levels remained stable throughout exercise and recovery, with no differences between trials. Noradrenaline and adrenaline increased during exercise in both visits and returned to baseline within 45 minutes post-exercise. Growth hormone also rose, peaking at the end of exercise and returning to baseline 60 minutes later in both visits. Cortisol levels were highest at baseline and secondary elevated in response to exercise, with the greatest rise observed at the end of the session in both the GLUC and NO-GLUC arms. Lactate concentrations were similar across visits and increased in response to exercise. These results are presented in figure 2 and 3.

3.4 Cardiorespiratory parameters

The exercise intensity was 61.4 ±14.4 and 59.8 ±12.2 %, $p=0.714$ during the GLUC and NO-GLUC arm respectively. Heart rate (HR) [112±11 vs 113±16 bpm, $p=0.8$], volume of oxygen uptake (VO_2) [1.06±0.28 vs 1.02±0.35 L/min, $p=0.4$], volume of carbon dioxide production (VCO_2) [0.94±0.23 vs 0.87±0.29 L/min, $p=0.1$], oxygen pulse (O_2 pulse) [9.5±2.3 vs 9.0±2.9 mL/min, $p=0.3$], and lipid oxidation () [0.21±0.11 vs 0.26±0.11 g/min, $p=0.1$], were all similar across trials. A higher respiratory exchange rate (ratio of VCO_2 divided by VO_2) [0.89±0.04 vs 0.85±0.02, $p=0.02$] and carbohydrate oxidation (g/min) [0.80±0.21 vs 0.63±0.19, $p=0.01$] rate were seen in the GLUC arm compared to the NO-GLUC arm.

4. DISCUSSION

This crossover study evaluated and compared glucose responses to spontaneous fasted exercise in T1D adults treated with MiniMed™ 780G AID with or without a glucagon injection on top of temporary target set

shortly before exercise. We demonstrated that mean glucose and glucose variability was slightly higher with glucagon but time in and above range were similar during the two trial days. Of special interest, we demonstrated that no hypoglycaemic episodes occurred during and after the exercise session regardless of the intervention arm. The absence of hypoglycaemia in the control arm was somewhat unexpected but may be explained by several factors. First, exercise was performed in fasting conditions with low levels of ambient insulin.[18] Second, the exercise session was performed in the morning, which also with other treatment modalities than AID reduces the risk of hypoglycaemia compared to afternoon exercise.[19,20] Third, the duration of the exercise session was relatively short, but previous studies of same duration and with the same AID system have shown that hypoglycaemia can occur, especially when exercise is performed without reducing meal insulin and setting the temporary target 90 minutes before the activity.[21,22]

The use of low-dose glucagon in our study did not improve the glycaemic profile around exercise. It led to a significantly higher mean, peak, AUC, and end-of-exercise plasma glucose concentrations, compared to the session without glucagon. Other studies have shown the same glucose-increasing effect of low-dose glucagon which in their cases prevented exercise induced hypoglycaemia. A study from Rickels et al.[23] examined strategies to prevent hypoglycaemia during 45-minute of fasted brisk walking in insulin pump-treated people with T1D. The authors compared a 150-µg glucagon injection, insulin pump basal rate reduction of 50 %, 40 g of glucose tablets, and no intervention. Both glucagon and glucose tablets significantly increased plasma glucose levels and prevented hypoglycaemia, while the control and insulin reduction groups experienced 5-6 hypoglycaemic events each. Studies with dual hormone closed-loop systems have demonstrated superior performances over an insulin-only system during exercise, with reduced time spent in hypoglycaemia.[24,25] In the present study there were no differences in any of the glucoregulatory hormones between the two conditions, besides an expected increase in the glucagon concentration in the GLUC-arm. Some of the findings are similar to the hormonal changes reported by Shetty et al.[26] who investigated the counterregulatory hormones during different exercise intensities.

We observed an elevated carbohydrate oxidation rate in the GLUC arm compared to the NO-GLUC arm. It could be explained by the increased glucose availability due to the increased hepatic glucose output after

administration of exogenous glucagon.[27] remains speculative whether the increased glucose availability prevents hypoglycaemia in other exercise settings than used in the present study, such as different exercise types, intensity, durations, and post-exercise observation period. With the knowledge that spontaneous exercise is challenging while using an AID pump, changing the time of day for exercise, and maintaining spontaneity, such as announcing the activity only 15 minutes beforehand, could be a more effective strategy for avoiding exercise-induced hypoglycaemia. Admittedly our study had some limitations including a small participant number, a non-randomised design, a relatively short exercise session and a homogeneous study population (relatively low HbA1c, hypoglycaemia awareness and long diabetes duration) in an in-clinic setting. It would be valuable to investigate in a randomised controlled trial glucagon's role in people treated with an AID system, across various exercise types, durations, and intensities, as well as at different times of day—comparing fasted morning exercise to afternoon exercise in both postprandial and non-postprandial states. Additionally, examining glucose metrics throughout the day and night in these different settings could prove insightful.

5. CONCLUSION

Our findings, in this proof-of-concept study, demonstrate that adults with T1D using the MiniMed™ 780G can perform fasted moderate-intensity exercise without hypoglycaemia if the temporary target was set shortly before. Therefore, glucagon was not needed for the prevention of hypoglycaemia related to fasted morning exercise with this specific AID system.

ACKNOWLEDGMENT

The authors express gratitude to the study participants and to Sandra Tawfik, who served as the biomedical scientist on the project. This project received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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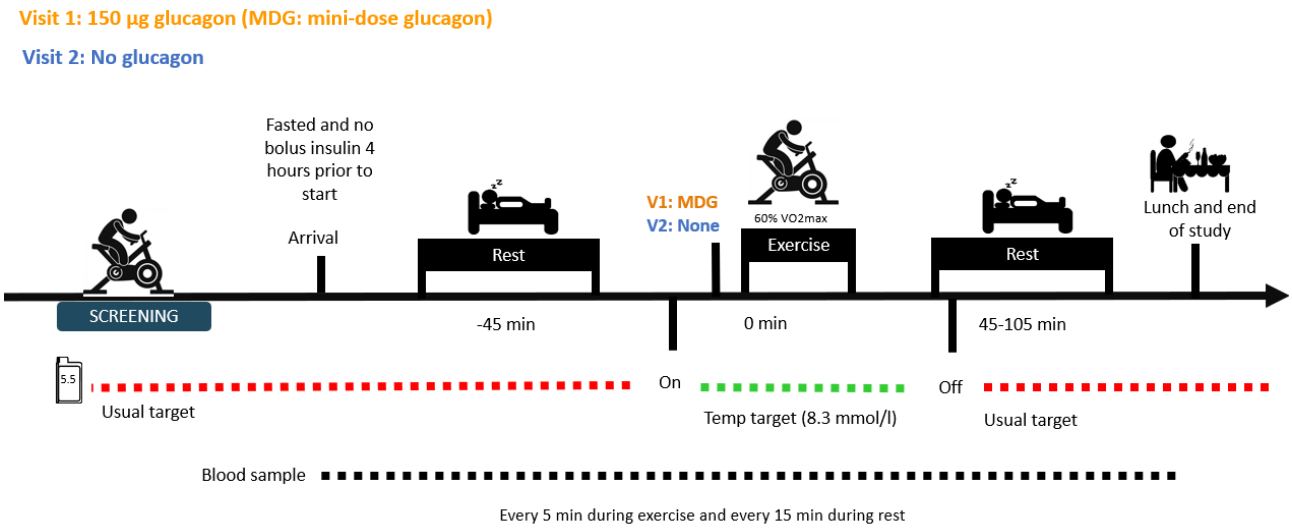
355 **FIGURE 1 Study design**



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357 Figure 1: Illustrating the study design and visits. Screening visit and two experimental visits were participants arrived at the research
358 facility after an overnight fast. Participants entered a resting phase of 45 min followed by 45 min of moderate intensity exercise at 60
359 % of VO2max. The study visit ended after a postexercise rest for 60 min. Temporary target was activated 15 min before and
360 deactivated 15 min after exercise. V1: MDG= mini dose of glucagon (150 µg), V2: None glucagon. Blood samples were taken every
361 5 to 15 min for measuring plasma glucose and lactate levels and later for hormonal biomarkers.

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TABLE 1 Baseline characteristics of the participants

Variable	Median	Range
Age (years)	50	42-67
Weight (kg)	79	55-103
Height (cm)	176	155-194
BMI (kg/m^2)	26	21-29
Type 1 diabetes duration (years)	22	14-44
Systolic BP (mmHg)	137	123-162
Diastolic BP (mmHg)	81	76-93
Heart rate (bpm)	65	46-84
HBA1c (mmol/mol)	55	47-69
HbA1c (%)	7.0	6.5-8.5
Total daily insulin dose (U)	42	22-84
TIR (%)	77	67-79
TBR (%)	1	1-10
Use of 780G pump (years)	1	0.1-6.9
$\dot{V}O_{2Peak}$ (ml/min/kg)	31	16-44

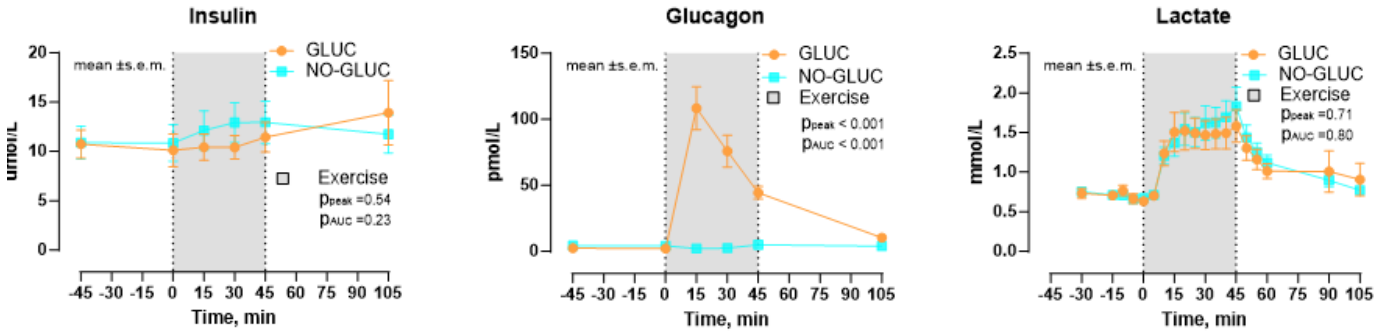
Table 1. Baseline, diabetes, and physical fitness characteristics of study participants. BMI: body mass index. BP: Blood pressure. HbA1c: Haemoglobin A1C, Time in range (TIR [plasma glucose: 3.9 – 10.0 mmol/l]), Time below range (TBR [plasma glucose <3.9 mmol/l]), $\dot{V}O_{2peak}$: the highest value of $\dot{V}O_2$ accomplished during rated exercise testing to exhaustion.

TABLE 2 Glucose outcomes for the combined exercise and post exercise period

	GLUC	NO-GLUC	p value	Test
TIR (%)	100 [91-100]	100 [100-100]	0.18	WSR
TITR (%)	44 [20-69]	97 [36-100]	0.10	WSR
TAR (%)	0 [0-0]	0 [0-0]	0.37	WSR
TBR (%)	0 [0-0]	0 [0-0]	1.00	WSR
Mean PG (mmol/l)	8.3±1.8	6.7±1.3	0.01*	TTEST
SD PG (mmol/l)	1.0 [0.7-1.2]	0.5 [0.3-0.6]	0.01*	WSR
CV PG (mmol/l)	12.3±4.3	7.3±2.5	<0.01*	TTEST
Nadir PG (mmol/l)	5.8 [5.2-6.9]	5.8 [4.9-6.5]	0.41	WSR
Peak PG (mmol/l)	9.3±1.8	7.5±1.4	0.01*	TTEST
PG0 (mmol/l)	6.3±1.8	6.8±1.1	0.54	RMA
PG45 (mmol/l)	8.9±1.8	6.2±1.6	<0.01*	RMA
PG105 (mmol/l)	7.5 [7.0-8.2]	6.7 [5.9-7.4]	0.07	RMA
Delta PG (mmol/l)	1.8 [0.9-2.3]	-0.6 [-0.8 to 0.1]	0.02*	WSR
AUC (mmol/l*min)	824.2 [782.3-941.2]	686.2 [612.6-821.1]	0.01*	Log TTEST
Incremental AUC (mmol/l*min)	27.6 [14.9-35.7]	-9.7 [-11.8 to -1.3]	<0.01*	WSR

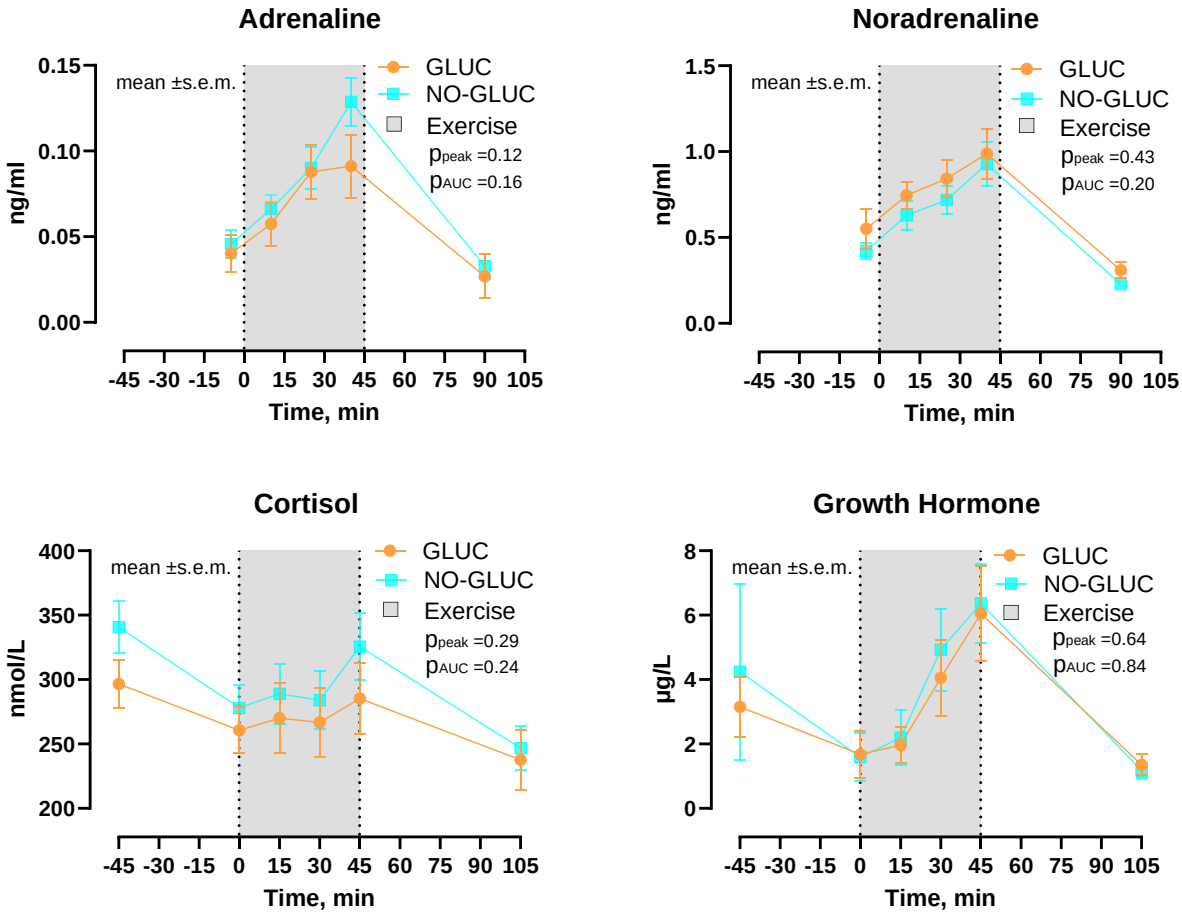
Data are presented as either Median [IQR] or Mean±SD. GLUC: Trial arm in which 150 µg of glucagon was administered at start of exercise, NO-GLUC: Trial arm without glucagon administration, TIR: Time In Range (plasma glucose: 3.9 – 10.0 mmol/l),, TITR: Time In Tight Range (plasma glucose: 3.9-7.8 mmol/l), TBR: Time Below Range (plasma glucose <3.9 mmol/l), TAR: Tim Above Range (plasma glucose >10.0 mmol/l), PG: Plasma Glucose, SD: Standard deviation, CV: Coefficient of variation, PG0: Plasma glucose at exercise onset, PG45: Plasma Glucose at min 45 i.e., at the end of exercise, PG105: Plasma Glucose at min 105 i.e., 60 minutes after the end of exercise, AUC: Areal Under the Curve, WSR: Wilcoxon Rank Test, Ttest: Paired T-test, RMA: Repeated Measurement ANOVA incl. Tukey adjustment for multiple comparison. *Significant difference $p<0.05$ between trial arms.

FIGURE 2 Insulin, glucagon, and lactate concentrations:



Data are represented in mean±s.e.m. GLUC: Trial arm in which 150 µg of glucagon was administered at start of exercise, NO-GLUC: Trial arm without glucagon administration. Peak: highest concentration measured. AUC: Areal Under the Curve. Significant difference $p<0.05$ between trial arms.

FIGURE 3 Adrenaline, noradrenaline, cortisol, and growth hormone concentrations:



Data are represented in mean±s.e.m. GLUC: Trial arm in which 150 µg of glucagon was administered at start of exercise, NO-GLUC: Trial arm without glucagon administration. Peak: highest concentration measured. AUC: Areal Under the Curve. Significant difference $p<0.05$ between trial arms.