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Pharmacokinetics of glucagon after intravenous, intraperitoneal and subcutaneous administration in a pig model

Ingrid Anna Teigen[1] | **Marte Kierulf Åm**[1] | **Sven Magnus Carlsen**[1,2] | **Sverre Christian Christiansen**[1,2]

[1]Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Endocrinology, St. Olav's Hospital, Trondheim University Hospital, Trondheim, Norway

Correspondence
Ingrid Anna Teigen, Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim N-7491, Norway.
Email: ingrid.a.teigen@ntnu.no

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Abstract

Introduction: There is increasing scientific evidence to substantiate using low-dose glucagon as a supplement to insulin therapy in artificial pancreata for diabetes mellitus type 1. The delivery of both these hormones intraperitoneally would mimic normal physiology. However, our knowledge of the pharmacological properties of glucagon after intraperitoneal administration is limited. This study compared the pharmacokinetics of glucagon after intraperitoneal, subcutaneous and intravenous administration and the pharmacodynamic effects of glucagon on glucose metabolism after intraperitoneal and subcutaneous administration in a pig model.

Materials and methods: Twelve pigs were included. Glucagon was administered intraperitoneally, subcutaneously and intravenously in a randomised order. Arterial samples were collected every 2–10 min for 150 min to determine plasma glucagon and blood glucose concentrations.

Results: The bioavailability of glucagon was significantly lower after intraperitoneal compared with subcutaneous administration with a median difference (95% confidence interval) of 13% (4–22). The effect of glucagon on glucose metabolism was equal after intraperitoneal and subcutaneous administration.

Conclusions: Intraperitoneal glucagon administration resulted in lower systemic glucagon exposure than subcutaneous administration without loss of efficiency. We interpret this as evidence of a major first-pass metabolism of glucagon in the liver.

KEYWORDS
artificial pancreas, diabetes mellitus type 1, glucagon, intraperitoneal infusion, pharmacokinetics

List of abbreviations: 95% CI, 95% confidence interval; $AUC_{0\text{-last}}$, area under the time-plasma concentration curve from time zero to time to last measurable concentration; C_{max}, maximum plasma concentration; DM1, diabetes mellitus type 1; HbA1c, glycated haemoglobin; IP, intraperitoneal; IQR, interquartile range; IV, intravenous; SC, subcutaneous; SD, standard deviation; $T_{1/2}$, plasma elimination half-life; T_{last}, time to last measurable concentration; T_{max}, time to maximum plasma concentration.

1 | INTRODUCTION

Glucagon increases hepatic glucose output in response to declining blood glucose concentrations in healthy individuals.[1] Most patients with diabetes mellitus type 1 (DM1) have a malfunctioning glucagon regulation, making them susceptible to hypoglycaemia.[2] Exogenous glucagon exerts the same pharmacodynamic effects on glucose metabolism as endogenous glucagon, and utilisation of low-dose glucagon to prevent and reverse mild hypoglycaemia has been investigated with some success, both as single subcutaneous (SC) injections and as part of SC artificial pancreata.[3–6]

After being secreted by the pancreas, insulin and glucagon are drained via the portal vein through the liver, the main action site for both hormones, before entering the systemic circulation. An artificial pancreas with intraperitoneal (IP) drug delivery could mimic this pathway, as drugs administered IP are absorbed predominantly by the splenic and mesenteric vessels that empty into the portal vein.[7] Unihormonal pumps administering solely insulin IP have demonstrated superiority over SC pumps in reducing glycated haemoglobin (HbA1c) and hypoglycaemic/hyperglycaemic episodes in humans.[8] In contrast, the pharmacological properties of IP delivered glucagon have only been studied in a few small-sized animal trials.[9–13]

To our knowledge, no comparative pharmacokinetic studies of intravenously (IV), SC, and IP delivered glucagon have been published. Thus, we investigated possible differences in glucagon pharmacokinetics based on the route of administration using a pig model. The primary aim of this study was to investigate the bioavailability of glucagon after IP administration, potentially providing evidence for our previously published hypothesis of extensive first-pass metabolism of glucagon in the liver.[12] Other relevant pharmacokinetic parameters, such as maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), time to last measurable concentration (T_{last}), plasma elimination half-life ($T_{1/2}$) and area under time-plasma concentration curve from time zero to T_{last} ($AUC_{0\text{-}last}$), after IV, IP and SC glucagon administration were evaluated as secondary outcomes. The pharmacodynamic effects of glucagon on glucose metabolism after IP and SC administration were also analysed.

2 | METHODS

2.1 | Study design

The study was preapproved by the Norwegian Food Safety Authorities (FOTS number 12948), and it complied with the "Norwegian Regulation on the Use of Animals in Research" and the 2010/63 EU directive on the "Protection of Animals Used for Scientific Purposes". The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[14]

We conducted a randomised, open-label, cross-over trial. To detect at least a 60% reduction in bioavailability (after IP compared with IV administration) with a power of 80% and an alpha value of 0.05, we included 12 female, non-diabetic farm pigs (*Sus scrofa domesticus*).

Each pig received three separate boluses of 1.5 μg/kg glucagon over a single study day: One bolus was administered in the left internal jugular vein, one bolus was delivered via a pump to the IP space in the upper left quadrant of the abdomen, and one bolus was administered via a pump to the SC adipose tissue behind the left ear. Both the IP and the SC boluses were delivered with an infusion speed of 100 μg/min. A 1.5 μg/kg glucagon dosage was chosen to correspond to a realistic dosage in artificial pancreata for humans, that is, 75–150 μg for individuals weighing 50–100 kg. The order of the bolus administrations was decided through a simple block randomisation procedure, with two pigs randomly allocated to each possible order.

2.2 | Animals and animal handling

All pigs were acquired from the same local supplier at approximately 12 weeks of age. Mean (standard deviation [SD]) weight was 45.4 (6.5) kg.

The animals were monitored continuously during the experiments. All surgical procedures, drug administrations and blood samplings were performed under general anaesthesia.

2.3 | Study procedure

2.3.1 | Premedication and anaesthesia

The pigs were premedicated before intubation with an intramuscular injection of 10 mg/kg azaperone (Separon vet.l®, Richter Pharma AG, Austria) and 10 mg/kg ketamine (Ketalar®, Pfizer AS, Norway) and an IV infusion of 1 mg atropine (Takeda AS, Asker, Norway). Anaesthesia was induced by an IV infusion of 150–250 μg fentanyl (Actavis Group, Hafnarfjörður, Iceland), 75–125 mg thiopental (VUAB Pharma AS, Roztoky, Czech Republic) and 150–250 mg ketamine. Anaesthesia was maintained by continuous IV infusion of 0.5 mg/kg/h midazolam (Accord Healthcare Limited, Middlesex, UK) and 7.5 μg/kg/h

fentanyl, together with continuous inhalation of 0.5%–2% isoflurane (Baxter AS, Oslo, Norway). An IV infusion of 2 g cephalothin (Villerton Invest SA, Luxembourg) was given as antibiotic prophylaxis immediately after establishing anaesthesia and repeated every third hour. The pigs were euthanised while still under general anaesthesia at the end of the study day with an IV infusion of 100 mg/kg phenobarbital (NAF, Apotek, Lørenskog, Norway).

2.3.2 | Suppression of endogenous glucagon and insulin secretion

To suppress endogenous glucagon and insulin secretion, the pigs received an initial IV bolus of 5 μg/kg octreotide (Sandostatin®, Novartis Europharm Limited, United Kingdom), followed by continuous IV infusion of 5 μg/kg/hour octreotide throughout the study day. Octreotide treatment was initiated 1 h before the first glucagon bolus. The concentration of porcine insulin was measured before and after every glucagon bolus to monitor the efficiency of suppression.

2.3.3 | Insulin and glucose infusions

The pigs received separate continuous IV infusions of 0.05 IU/kg/hour insulin aspart (NovoRapid®, Novo Nordisk AS, Denmark) and 20% glucose solution (Glucos B. Braun®, Braun, Germany) throughout the study day to prevent glycogen depletion. Before each glucagon bolus, blood glucose concentration was titrated to a normoglycaemic target concentration of 4–5 mmol/L by adjusting the glucose infusion rate. According to the study protocol, the blood glucose concentration had to be stable (no more than 0.2 mmol/L variations) before every glucagon administration, without any glucose infusion rate adjustments for 20 min. The glucose infusion rate was also kept stable for the first 60 min after glucagon administration to monitor the effects on glucose metabolism.

2.3.4 | Blood sampling

Arterial blood samples were drawn immediately before each glucagon bolus, and subsequently every 2 min for the first 40 min, every 5 min for the next 60 min, and every 10 min for the remaining 50 min. We set the observation time to 150 min to ensure a wash-out period of more than five estimated $T_{1/2}$ between each bolus.[12,15]

2.3.5 | Sample handling and analysis

Arterial blood glucose concentrations were analysed immediately after collection with a Radiometer ABL 800 FLEX blood gas analyser. The inter-assay coefficient of variation was below 5%.

After centrifugation, arterial plasma samples for hormone analyses were stored at −18°C for the duration of the experiment, and later at −80°C until analysis. Glucagon concentrations were measured using Glucagon ELISA kits (Mercodia, Uppsala, Sweden), and porcine insulin concentrations were measured using Porcine insulin ELISA kits (Mercodia, Uppsala, Sweden). Both glucagon and porcine insulin samples were run in singles. All glucagon and porcine insulin ELISA kits were from the same batches. The analyses were performed in the same setup by the same engineer. Both intra-assay and inter-assay variation were below 10% for glucagon and below 5% for porcine insulin.

2.4 | Data analysis

2.4.1 | Pharmacokinetic analysis

C_{max}, T_{max} and T_{last} were obtained directly from the measured glucagon concentrations in plasma after correcting for baseline concentrations. $T_{1/2}$ and $AUC_{0\text{-last}}$ were estimated using Simbiology in MATLAB version R2020B.[16] The terminal rate constant, describing the decrease of the log-concentration of glucagon, was calculated by applying a best-fit linear regression to the terminal portion of the curve. $T_{1/2}$ was calculated as $\frac{\ln 2}{\text{terminal rate constant}}$. $AUC_{0\text{-last}}$ was calculated by using the linear trapezoidal method.

2.4.2 | Pharmacodynamic analysis

The glucose concentrations were baseline-corrected. Only IP and SC administrations were included in the pharmacodynamic analyses, as this was done primarily to verify results from a previous, smaller pig study.[13] Furthermore, the IV route is currently only used in emergency and in-hospital settings and is not feasible for artificial pancreata.

2.4.3 | Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.[17] Non-parametric tests were chosen because of

the small sample size and skewed distribution of some variables.

Medians and interquartile ranges (IQR) plus means and SD of T_{max}, C_{max}, $AUC_{0\text{-last}}$, $T_{1/2}$ and T_{last} were calculated. Possible differences in T_{max}, C_{max}, $AUC_{0\text{-last}}$, $T_{1/2}$ and T_{last} in relation to administration routes were examined using the Friedman test. Correction for multiple analyses was performed using Dunn's multiple comparison test.

Bioavailability was assessed by comparing $AUC_{0\text{-last}}$ after IV administration with $AUC_{0\text{-last}}$ after IP and SC delivery. Median and mean bioavailability for the IP and SC route was calculated. Possible differences in bioavailability after IP and SC administration were examined using the Wilcoxon matched-pairs signed-rank test.

Differences in blood glucose concentrations over time after IP and SC administration was analysed using a mixed-effects analysis with time and administration route as fixed effects and the subject number as a random effect. Correction for multiple analyses was performed using Šídák's multiple comparison test.

3 | RESULTS

3.1 | Exclusions

The following pigs were excluded from the analyses to maintain pairing:

Pig 1 was excluded from all analyses because we suspected that the SC catheter had not penetrated the skin properly upon removing it. No increase in blood glucose concentration was observed after this bolus, and only a minimal increase in plasma glucagon concentration was noted between 38 and 45 min after drug administration, which could be due to percutaneous absorption.

Pig 11 was excluded from only the pharmacodynamic analyses because the glucose infusion set was blocked shortly before SC glucagon administration, which led to a marked drop in the blood glucose concentration at baseline. It was kept in the pharmacokinetic analyses as we regard it unlikely that this temporary drop in glucose concentration, which was corrected within the following 2 min, would have affected glucagon metabolism.

Pig 12 was excluded from all analyses as it had abdominal adhesions and gross vascular anomalies compatible with previously advanced peritonitis, which would likely affect IP drug absorption. A veterinary surgeon confirmed the diagnosis on site.

3.2 | Suppression of endogenous insulin and glucagon secretion

The plasma glucagon concentration was below the quantification cut-off limit of 1.95 pmol/L before the first glucagon bolus in all included pigs and porcine insulin was below the quantification cut-off of 2.3 mU/L in all included pigs, except one (Pig 5, 3.6 mU/L). The porcine insulin concentrations remained low throughout the experiments and were below the quantification cut-off limit in 53 out of 60 samples in the included pigs. The mean (SD) porcine insulin concentration was 3.8 (0.8) mU/L in the remaining seven samples.

3.3 | Pharmacokinetic parameters

Ten pigs were included in the pharmacokinetic analyses. Pharmacokinetic findings are summarised in Table 1. Mean glucagon concentration with their respective 95% confidence intervals (95% CI) at every time point for each administration route is presented in Figures 1 and 2. Plasma glucagon concentration over time after IP and SC administration for each pig is presented in Figure 3. The concentrations were baseline-corrected. Plasma glucagon concentration was below the quantification cut-off limit of 1.95 pmol/L at baseline in 24 of 30 included boluses. For these boluses, the baseline concentration was set at 0 pmol/L. The mean baseline (SD) concentration was 4.0 (1.7) pmol/L in the six remaining boluses.

Glucagon concentrations increased rapidly in plasma regardless of administration route. Median (IQR) T_{max} was 2 (2–2), 13 (10–18) and 10 (8–12) min after IV, IP and SC administration, respectively, and mean (SD) T_{max} was 2 (0), 15 (8) and 10 (4) min. T_{max} was significantly lower with IV administration as compared with both IP and SC administration (p value < 0.001 and <0.01, respectively). There was no significant difference in T_{max} between IP and SC administration (p value > 0.99).

Median (IQR) C_{max} was 1735 (393–1965), 7 (5–10) and 31 (14–59) pmol/L after IV, IP and SC administration, respectively, and mean (SD) C_{max} was 1372 (790), 8 (6) and 37 (24) pmol/L. C_{max} was significantly higher with IV administration than with IP administration (p value < 0.0001). No significant difference in C_{max} was observed when we compared IV and SC or IP and SC administration (p value 0.08 for both comparisons).

Median (IQR) $AUC_{0\text{-last}}$ was 7670 (2389–8600), 163 (87–235) and 991 (426–1359) pmol/L/min after IV, IP and SC administration, respectively, and mean (SD) $AUC_{0\text{-last}}$ was 6476 (3383), 161 (79) and 961 (540) pmol/L/min. $AUC_{0\text{-last}}$ was significantly larger with IV administration than with IP administration

TABLE 1 Pharmacokinetic parameters

Pharmacokinetic Parameter	Intravenous Administration	Intraperitoneal Administration	Subcutaneous Administration
Time to maximum plasma concentration (minutes)	2 (2–2)	13 (10–18)	10 (8–12)
Maximum plasma concentration (pmol/L)	1735 (393–1965)	7 (5–10)	31 (14–59)
Area under the time-plasma concentration curve from time zero to time to last measurable concentration (pmol/L/min)	7670 (2389–8600)	163 (87–235)	991 (426–1359)
Plasma elimination half-life (minutes)[a]	6 (3–8)	10 (8–12)	15 (9–18)
Time to last measurable concentration (minutes)	48 (44–61)	41 (32–59)	68 (50–81)
Bioavailability (percentage)[b]	—	3 (2–5)	16 (9–22)

Note: Data are reported as medians (interquartile range) when not stated otherwise.
[a]7 animals included.
[b]Values are stated as median (95% confidence interval).

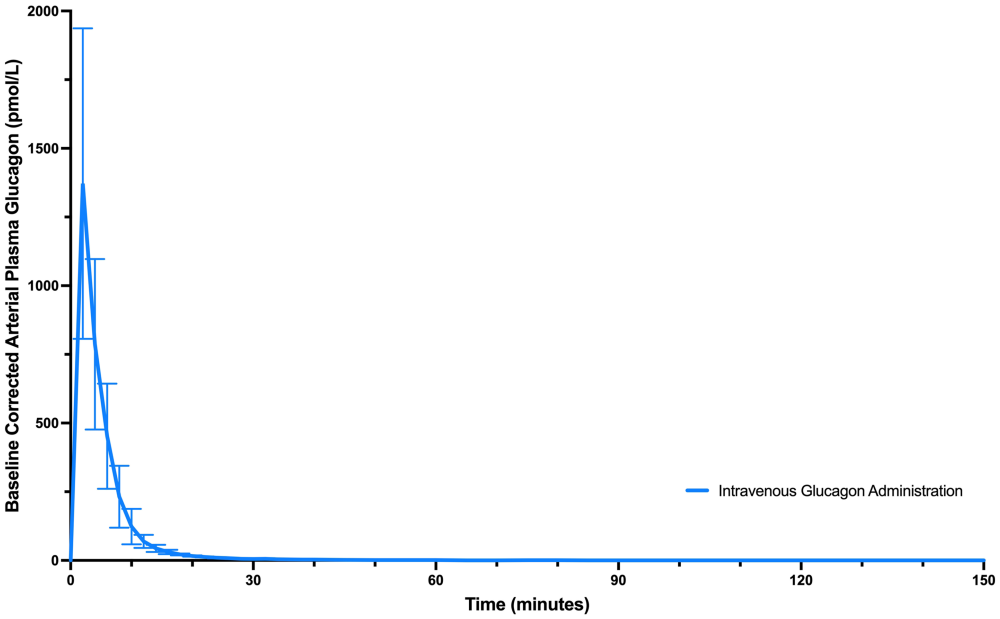

FIGURE 1 Mean glucagon concentrations in arterial plasma (with 95% confidence interval [CI] error bars) over time after intravenous administration

(p value < 0.0001). No significant difference in $AUC_{0\text{-last}}$ was observed when we compared IV and SC or IP and SC administration (p value 0.08 for both comparisons).

Irregular elimination patterns after IP administration of glucagon in Pigs 2 and 3 and after SC administration of glucagon in Pig 5 prevented reliable calculation of $T_{1/2}$ from these. Therefore, these three pigs were excluded from the $T_{1/2}$ analysis. Median (IQR) $T_{1/2}$ after IV, IP and SC administration was 6 (3–8), 10 (8–12) and 15 (9–18) min, respectively, whereas the mean (SD) was 9 (10), 13 (8) and 13 (5) min. No significant differences between administration routes were observed (p value > 0.10 for all comparisons).

Plasma glucagon concentrations returned to baseline within 150 min after all boluses except after IV and SC administration in Pig 5. Median (IQR) T_{last} was 48 (44–61), 41 (32–59) and 68 (50–81) min, respectively, whereas mean (SD) T_{last} was 59 (34), 45 (20) and 74 (30) min. No significant differences between administration routes were observed (p value > 0.10 for all comparisons).

Median (IQR) bioavailability was 3% (2–5) and 16% (9–22) with IP and SC administration, respectively, and the mean (SD) bioavailability was 3% (2) and 22% (26). The bioavailability of glucagon was significantly lower after IP compared with SC administration (p value 0.002), with a median difference (95% CI) of 13% (4–22).

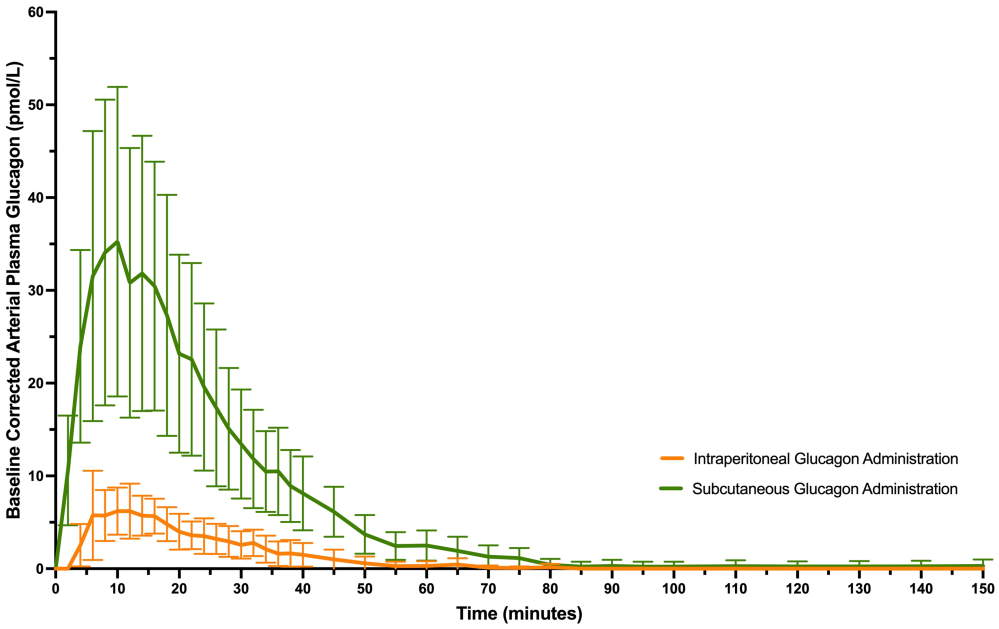

FIGURE 2 Mean glucagon concentrations in arterial plasma (with 95% confidence interval [CI] error bars) over time after intraperitoneal and subcutaneous administration

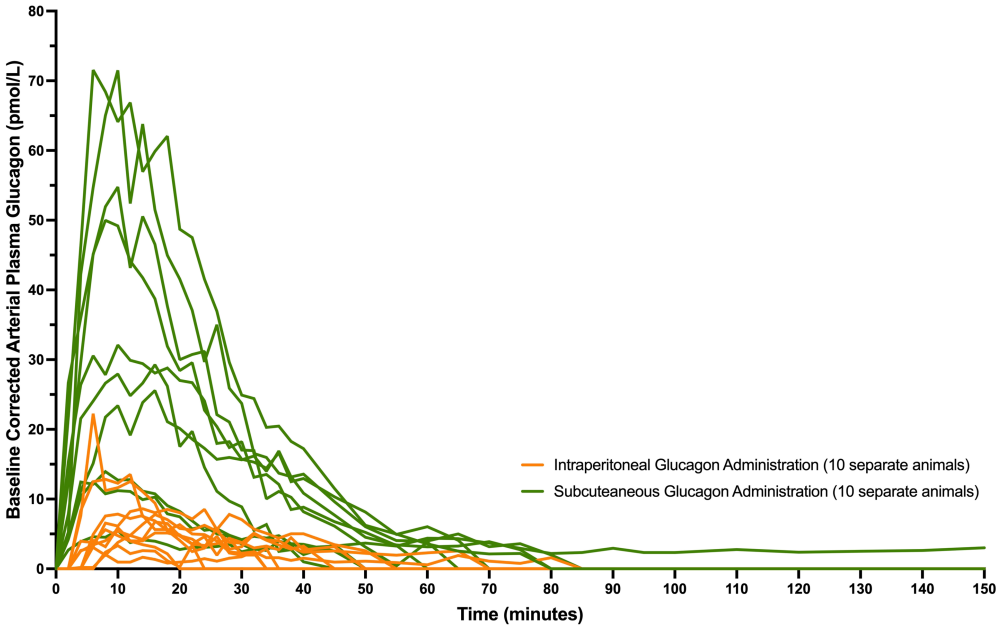

FIGURE 3 Glucagon concentrations in arterial plasma over time for all included pigs after intraperitoneal and subcutaneous administration

The bioavailability remained significantly different (p value 0.004) also after excluding Pig 11, which was excluded from the pharmacodynamic analysis, with a median difference (95% CI) of 13% (4–22).

3.4 | Pharmacodynamic parameters

Nine pigs were included in the pharmacodynamic analyses. Mean blood glucose concentrations with their respective 95% CI between 0 and 60 min after IP and SC glucagon administration are presented in Figure 4. The concentrations were baseline corrected. The mean (SD) blood glucose concentration at baseline was 4.6 (0.3) mmol/L for the IP boluses and 4.3 (0.4) mmol/L for the SC boluses.

There was no significant difference (95% CI including 0 and p value > 0.90) in mean blood glucose concentration after IP and SC administration at any point in time (Figure 3).

4 | DISCUSSION

Despite having significantly lower bioavailability, IP administered glucagon exerted comparable effects on

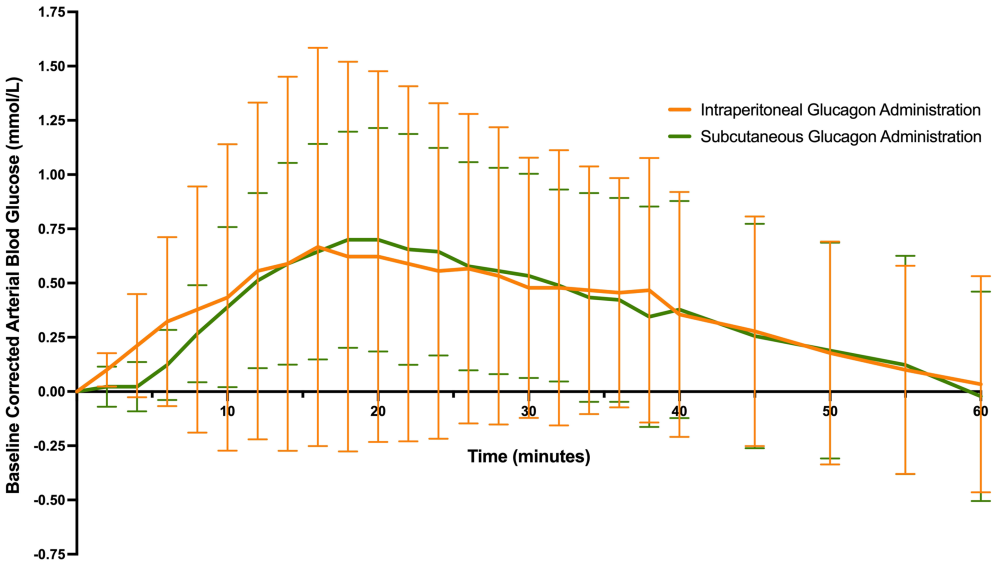

FIGURE 4 Mean glucose concentrations in arterial blood (with 95% confidence interval [CI] error bars) over time after intraperitoneal and subcutaneous administration

glucose metabolism as SC administered glucagon in the present study. A major first-pass metabolism of glucagon in the liver is the most likely explanation for this observation. In theory, this could be advantageous because high systemic concentrations of glucagon are associated with more pronounced adverse reactions.[18]

The observed equivalent effect on glucose metabolism after IP and SC glucagon administration contradicts the findings of two animal studies previously performed by our research group, Artificial Pancreas Trondheim. In those studies, IP administration was associated with either a faster[9] or a more pronounced[13] glucose response. In the previous studies, we used smaller glucagon doses, and it is possible that we saturated the liver's ability to increase glucose output regardless of administration route by administering a larger dose in the present study. However, the former pig study, which demonstrated superior glucose elevating effect through IP administration, only detected this difference upon excluding four of 10 originally included pigs. Moreover, there was no difference in total glucose elevation over time, as measured by AUC.[13] This indicates that there might not be a genuine difference in response between the two administration routes. A study examining the effects of glucagon at different doses is needed to investigate this further.

In the same pig study mentioned above, blood glucose concentrations increased more than in the present study, despite using a smaller glucagon dosage (0.6 μg/kg).[13] We believe this could be because the pigs in the previous study received no insulin during the study day, as insulin and glucagon exert antagonistic effects on glucose metabolism in the liver. Although an artificial pancreas would likely reduce and ultimately discontinue insulin infusion as a response to hypoglycaemia, a situation where there is no circulating insulin is highly unrealistic. As such, the results from our present study could be more translatable to a real-life scenario.

As expected, the initial peak in plasma glucagon concentration occurs later after SC and IP administration compared with IV administration. The first sample was drawn 2 min after the drug was administered. Therefore, the exact values of C_{max} and T_{max} after IV infusion are probably unknown. Following IP and SC administration, absorption is rapid, and T_{max} is reached at a similar pace.

There was no significant difference in C_{max} and $AUC_{0\text{-last}}$ between IP and SC administration. However, there was a tendency towards a difference, with a p value of 0.08 for both parameters after correcting for multiple comparisons. It is possible that the study became underpowered to detect any difference, as we had to exclude two pigs from the analyses, and that a larger study would have conveyed a different result.

There were no significant differences in $T_{1/2}$ between the different routes of administration. However, this result should be interpreted with caution because a stable elimination of glucagon from plasma was not observed after three administrations, which prevented a reasonable calculation of $T_{1/2}$ from these boluses. This could possibly be due to prolonged absorption of glucagon from the administration site, temporary failure of suppression of endogenous glucagon secretion or interindividual variation in metabolism.

Reduced systemic drug exposure after IP administration compared with SC administration could make the IP route preferable in a bihormonal artificial pancreas. However, IP drug delivery is invasive and associated with a high risk of serious complications. As such, it would only be an acceptable route of administration if it were to prove greatly advantageous in terms of glucose control.

Continuous IP insulin infusion is associated with lower HbA1c and fewer episodes of hyperglycaemia and hypoglycaemia than continuous SC insulin infusion in patients with DM1. Notably, the circulating insulin concentrations are also significantly reduced after IP administration, probably because of the large first-pass metabolism of insulin in the liver.[19] As hyperinsulinemia has been linked to the pathogenesis of cardiovascular diseases, reducing insulin in systemic circulation could hypothetically result in improved long-term health outcomes for patients with DM1.

4.1 | Limitations

Local degradation or formation of drug-containing loculaments in the IP cavity may have reduced the absorption of glucagon. Direct drug infusion into the portal vein would likely give a more precise estimate of first-pass metabolism. However, because this is not a viable treatment option in humans, we relied on IP administration to gain information on other relevant aspects for the possible use of glucagon in an IP artificial pancreas.

Pigs undergoing prolonged anaesthesia may accumulate IP fluid, which possibly could influence drug absorption from the IP cavity.[20] Theoretically, anaesthetic drugs and general anaesthesia may also lead to alterations in the pharmacokinetics and pharmacodynamics of glucagon. Our decision to perform these experiments under general anaesthesia was based on considerations for animal welfare.

The pigs received three glucagon boluses in one study day. Reduced effectiveness of consecutive doses of glucagon has been observed in healthy volunteers, possibly because of glycogen depletion.[21] However, other studies on human subjects with DM1, and a previous pig study conducted by our group, detected no association between glucose response and bolus number.[13,22,23] In the present study, the pigs received continuous infusions of insulin aspart and glucose solution to promote hepatic glycogenesis and reduce the risk of glycogen depletion.

A considerable limitation of the study is that both glucagon and porcine insulin samples were run in singles, which reduces the precision. However, we regarded this as acceptable, because the short time interval between samples made it possible to detect any marked outliers that needed to be reanalysed.

This study had a small sample size, with only 12 pigs. Some pigs had to be excluded from the analyses, which reduced the number even further. However, the main results regarding the bioavailability of IP glucagon are consistent and probably unaffected by this limitation.

5 | CONCLUSIONS

This study demonstrates that IP administered glucagon has lower bioavailability than SC administered glucagon, although the effect on glucose metabolism is equivalent. These results are compatible with a major first-pass metabolism of glucagon in the liver. Lower systemic drug concentrations could, in theory, lead to fewer adverse reactions. However, the SC route carries a much lower risk of complications and would doubtlessly be preferred for most patients with DM1. Nonetheless, a bihormonal IP artificial pancreas could be a possible treatment option for a subgroup of patients who may benefit from IP insulin administration.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data supporting the findings are available from the corresponding author upon reasonable request.

ORCID

Ingrid Anna Teigen https://orcid.org/0000-0002-2389-4444

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