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# Pharmacodynamics and Stability of Subcutaneously Infused Glucagon in a Type 1 Diabetic Swine Model In Vivo

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## ABSTRACT

**Background:** The objective of this study was to determine the in vivo pharmacodynamics of glucagon and to test its glycemic effect over days by assessing its time course of activity and potency in a type 1 diabetic swine model.

**Methods:** Individual experiments were conducted in different pigs using glucagon preparations that were reconstituted on different days and stored at room temperature or near body temperature before usage. All experiments involved a subcutaneous bolus of glucagon to counter impending hypoglycemia induced by an earlier bolus of fast-acting insulin. Frequent blood-glucose measurements, using a standard in vitro hand-held meter, were taken during each experiment to track variations in blood-glucose concentration.

**Results:** Significant glucagon action was observed as early as 5 min after administration, as evidenced by an effective halt to declining blood glucose and a subsequent twofold rise in blood glucose after ~20 min. Results also indicate that the consumption of glucagon from the subcutaneous depot is substantially faster than that of fast-acting insulin. Furthermore, no significant depreciation was observed in glucagon efficacy across aging preparations as old as 7 days.

**Conclusions:** These results suggest profound utility of subcutaneous glucagon in a closed-loop glucose control system, especially since glucagon would provide an effective safeguarding measure to stave off impending hypoglycemia, an application where the rapid effect of subcutaneous glucagon is both serendipitous and essential. Despite the notion that the stability of glucagon in solution at room temperature is inferior to that of fast-acting insulin, its subcutaneous administration has promising prospects for long-term closed-loop ambulatory care.

## INTRODUCTION

GLUCAGON IS A PARENTERAL HORMONE that plays a vital physiological role, namely, working to raise the blood-glucose (BG) concentration when the body experiences a shortage in glucose supply and/or to curb or restrain impending hypoglycemia. Although other hormones can occasionally have analogous effects vis-à-vis BG, such as thyroid, cortisol, epinephrine, and growth hormone, glucagon uniquely inhibits insulin secretion and is the hormone that is usually used by the body to raise BG, specifically by liberating glu-

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cose supplies from the liver and muscle cells (glycogenolysis) or by stimulating the generation of glucose in the liver from fats and proteins (gluconeogenesis). The objectives of this study are to test, quantitatively and qualitatively, the in vivo potency and time course of activity of subcutaneously administered glucagon, and to reveal any variations in these quantities with aging glucagon preparations that are maintained in solution at room temperature (~20°C) or near body temperature. These in vivo pharmacodynamics have not been thoroughly studied to date, especially in the context of small subcutaneous (SC) doses of glucagon, as opposed to intravenous (IV) or intramuscular doses, which are commonly used as a rescue measure during severe hypoglycemic episodes and seizures. This essentially denied glucagon a great potential utility in closed-loop glucose control, for which stability and short-term degeneration issues, if any, are particularly important. The prevailing notion of glucagon's inferior stability relative to that of insulin, however, is apparently exaggerated, since the context in which glucagon would be used can render such inferiorities inconsequential and therefore irrelevant. Furthermore, with an effective closed-loop system, the subject remains in tight metabolic regulation, and approaches the average person's ability to recover spent glycogen stores in the liver within 24–48 h, which in turn can last ~12–14 h before depletion around periods of no food intake or activities of high intensity.

## MATERIALS AND METHODS

All experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Boston University, Boston, MA (protocol number AN-14568).

### *Drugs and supplies*

Fast-acting insulin lispro (Humalog®, Eli Lilly, Indianapolis, IN) (from standard U-100 vials) and glucagon emergency kits (Eli Lilly) (1 g of lyophilized glucagons and 1 mL of diluent) were used. For SC delivery of glucagon, a customized Bluetooth-enabled Deltec Cozmo® infusion pump (Smiths Medical MD, Inc., St. Paul, MN) was used. The pump's cartridge can hold 300 U of either drug and can deliver variable bolus quantities in less than 1 min. For measurements of BG, an in vitro hand-held whole-BG meter was used (One Touch® Ultra, LifeScan, a division of Johnson and Johnson, Milpitas, CA), with blood samples obtained from ear pricks (capillary BG) or venous blood draws.

### *Animals*

Swine are prominently known as excellent humanoid models that can provide a unique testing platform for glycemic-related experiments. First, swine are omnivores with diets and metabolic properties that are similar to those of the human. Furthermore, skin similarities, in terms of lipid content and SC-tissue structure, and a similar absorption rate into plasma of SC doses of both biosynthetic human insulin and man-made fast-acting insulin aspart, make swine a particularly good humanoid model in the context of the SC drug dosing.[1] Such resemblance is not observed in rats and dogs, both of which absorb SC insulin injections faster and with no noticeable discrimination in the rate of absorption between human insulin and fast-acting insulin aspart.[1]

In light of these similarities between pig and human, a diabetic swine model was used in our experiments. Note that our experiments could not be conducted in healthy swine, since such subjects have the ability to regulate and maintain their BG in range by secreting endogenous insulin. (Although subjects with type 1 diabetes have the ability to secrete glucagon, the secretion process is somewhat compromised,[2] primarily because the loss of beta-cells constitutes loss of the physiological BG sensing mechanism.) Endogenous insulin secretion would certainly interfere with our experimental results and would, in the least, act as a confounding factor in assessing the overall potency of exogenous glucagon, since endogenous insulin would work against exogenous glucagon action, thereby significantly attenuating its effect in raising BG. Our diabetic swine model shows compelling symptoms that resemble type 1 diabetes-like pathology, including elevated fasting BG levels (on average, 319 mg/dL for the diabetic model

vs. 34 mg/dL in healthy swine) and elevated postprandial BG levels (on average, 533 mg/dL for the diabetic model vs. 51 mg/dL in healthy swine).[3,4]

*Induction of type 1 diabetes in swine*

Male Yorkshire swine, weighing ~15 kg each (10–12 weeks of age and weaned), were obtained from a breeder and housed together. After a few days of acclimation, the time during which the pigs were checked for parasites or disease, and their overall health was confirmed to be in good status, a type 1 diabetes-like pathology was induced. The induction is achieved using beta-cell cytotoxin streptozotocin (STZ) doses of 50–70 mg/kg, mixed in 10 mL of a sodium citrate buffer solution per 1 g of STZ, with pH adjusted to 4.5 using glacial acetic acid, and administered intravenously to each pig (0.5 mL of solution per 1 kg of body weight) via ear-vein catheters once a day for three consecutive days. To carry out the injection procedure, the pigs were completely sedated with an intramuscular injection of Telazol® (a mixture of tiletamine and zolazepam, Fort Dodge, Fort Dodge, IA) (6 mg/kg) and xylazine (3 mg/kg) mixed in 3–5 mL of saline, and their ears were subsequently swabbed with 70% isopropyl alcohol prior to venipuncture. Ears were alternated on each day of STZ administration, using a different vein, or a different location on a used vein for successive doses. Pigs were ready for experiments to commence about 2 weeks after injection with STZ.

*IV catheterization*

An ear-vein catheter line was established in each experiment to administer a continuous IV drip of saline, in order to prevent dehydration, to administer IV doses of insulin, and to sample BG from venous draws. The catheter insertion site was located near the extremity of the ear, which was first shaved and scrubbed with antiseptic. Regular BG samples were taken from the catheter (every 5–10 min).

*Infusion-set insertion*

Before the infusion sets were fixed onto a pig, the insertion site area was shaved with regular razors, scrubbed with antiseptic, and covered with sterile self-adhesive occlusive dressing (Tegaderm™ Transparent IV, 3M, St. Paul, MN). Typical insertion sites were on the pig's back, around the shoulder area. Commercially available infusion sets (Silhouette®, Medtronic MiniMed, Northridge, CA) were inserted with a thin trochar using a spring-loaded insertion device. Once the infusion set was inserted, the trochar was withdrawn and discarded. The soft tapered flexible cannula (~1.5 cm long) that remained in the SC tissue was inserted at an angle of ~30° and penetrated ~0.5 cm below the skin.

*Glucagon experiments*

All experiments were conducted in anesthetized pigs and lasted 2–3 h. Initial sedation was achieved with an intramuscular injection of Telazol (6 mg/kg) mixed in 3–5 mL of saline and administered while the pigs were inside their stalls. General anesthesia was maintained using ~2.5% isoflurane in conjunction with an oxygen flow rate of 2 L/min administered through a nose cone. Several glucagon solution samples were prepared ahead of time and maintained at room temperature (~20°C) or near body temperature (stored in a pouch worn on the back of the pig) prior to each experiment. Pigs were allowed sufficient recess time between experiments in order to restore their livers' glycogen stores. AU1

An initial IV insulin dose of ~2–5 U was administered (since our objective was to study the pharmacodynamics of SC glucagon, and not that of insulin, insulin was administered intravenously in order to expedite its effect in lowering BG), which constitutes a safe yet effective dose for the range of pig weights considered. On the other hand, the infusion pump was used for the subsequent SC administration of glucagon (typically ~5–10 U), which was initiated after a substantial decrease in BG concentration had occurred. In every case, glucagon was administered before hypoglycemia occurred, and BG was monitored to confirm effective glucagon action.

## RESULTS

Figures 1–4 show paired plots of BG and the corresponding doses of insulin and glucagon. F1–4

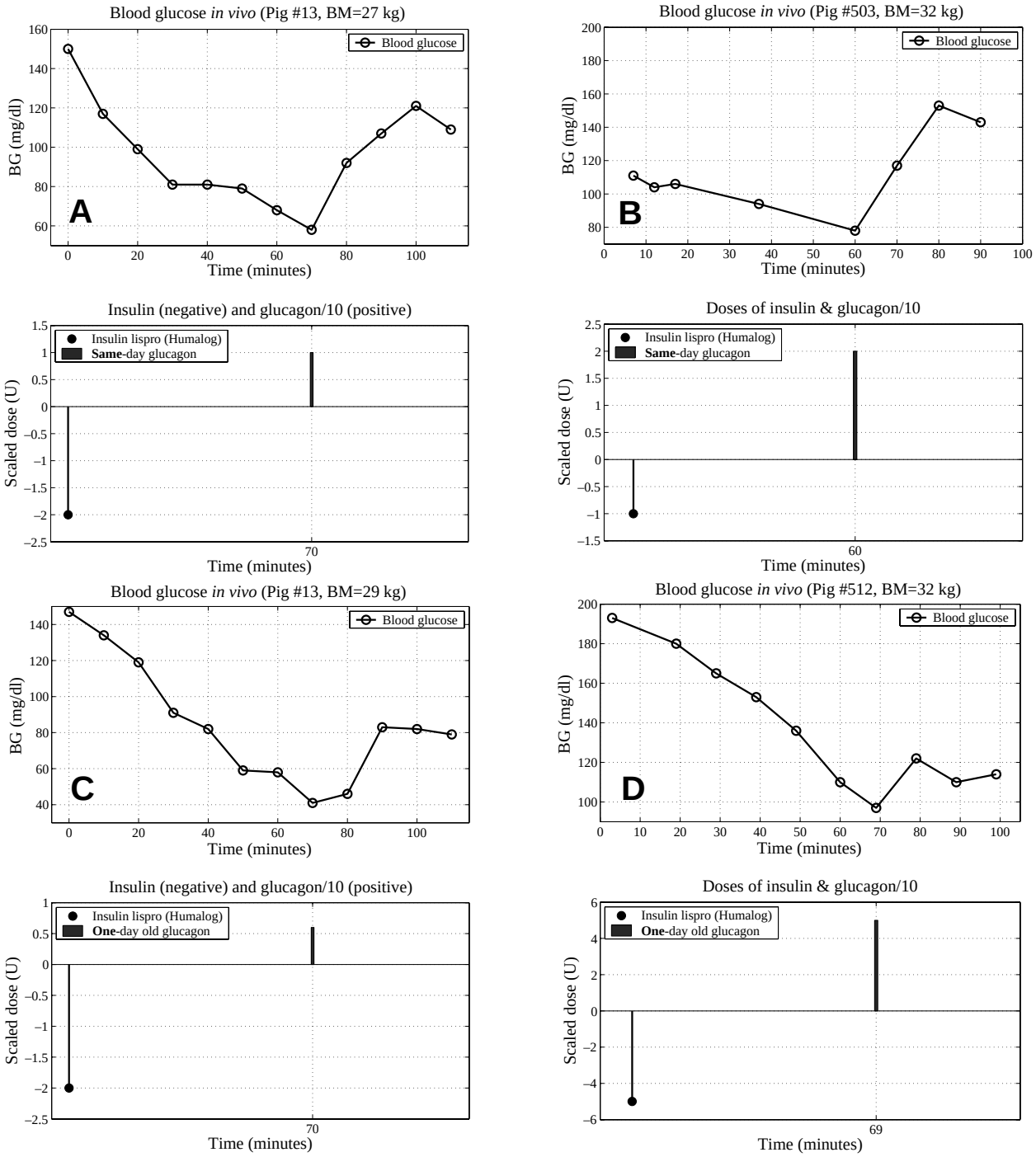


**FIG. 1.** (**A–D**) Four similar pairs of panels from four different experiments, with the **top panel** in each pair showing BG in vivo, denoted by circles, and the **bottom panel** showing the corresponding insulin bolus (stem, negative) later followed by a SC glucagon bolus (bar, positive). Glucagon preparations for the four experiments varied in age, as indicated in the panels. Six different pigs were used for the 15 experiments shown here and in Figs. 2–4.

Figures 1–4, top panels, depict in vivo BG, and bottom panels show a stem plot of the corresponding dose of insulin and a bar plot of the subsequent SC dose of glucagon. Each pair corresponds to a different experiment that used a glucagon preparation of a particular age. Specifically, up to and including 7-day-old glucagon preparations were used in a total of

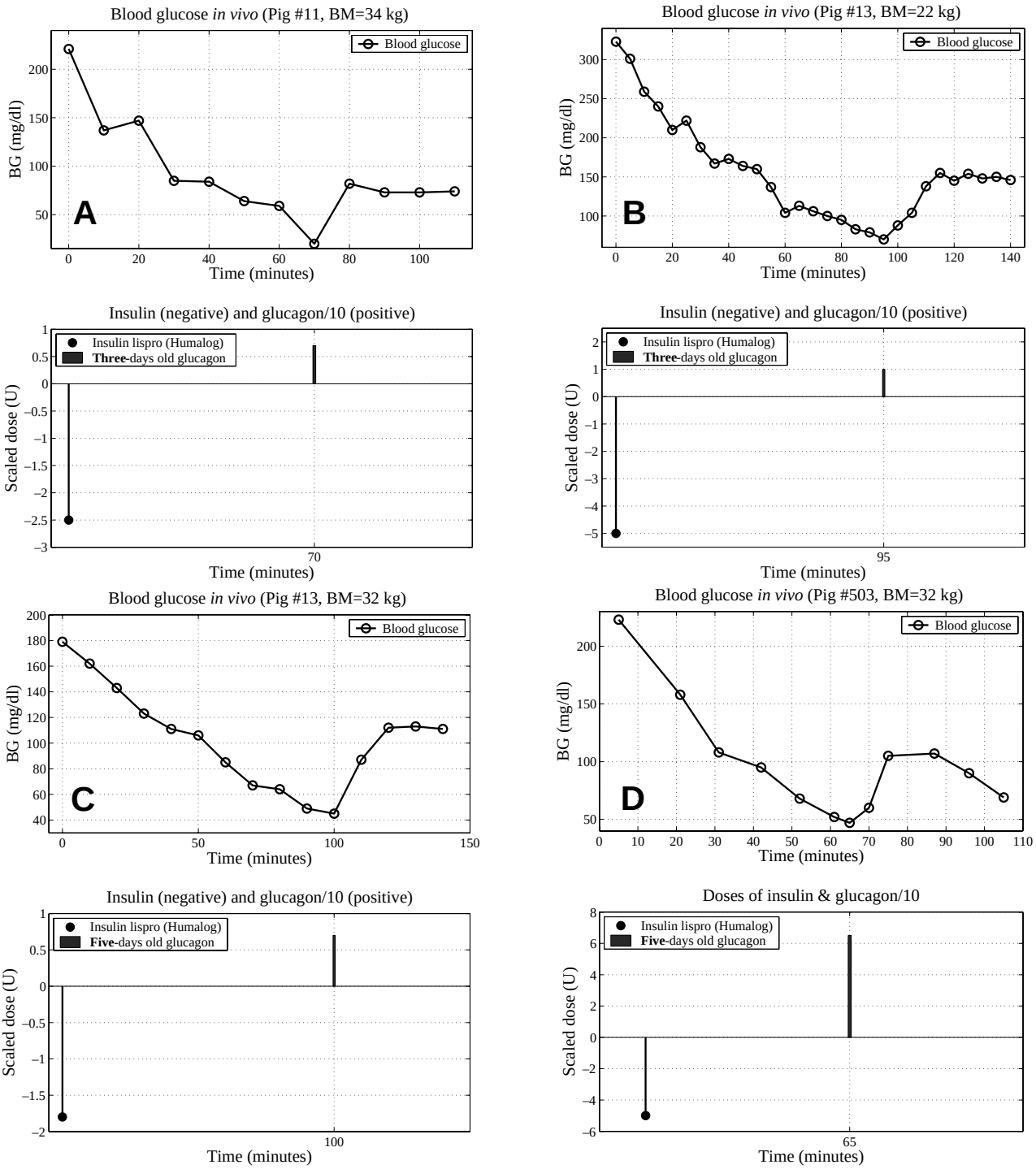


**FIG. 2.** Same explanation as in Figure 1.

15 experiments, and a total of six diabetic pigs were involved. Experiments were repeated in different pigs to provide confidence in glucagon's potency. The experiments involving Pig #31 and Pig #36 (see Figs. 3 and 4) used glucagon preparations that were stored in a pouch worn on the back of the pig for 7 days prior to each experiment. Storing the glucagon in this way accounts for the effects of body temperature on glucagon stability, whereas the other experiments used glucagon preparations stored at room temperature.

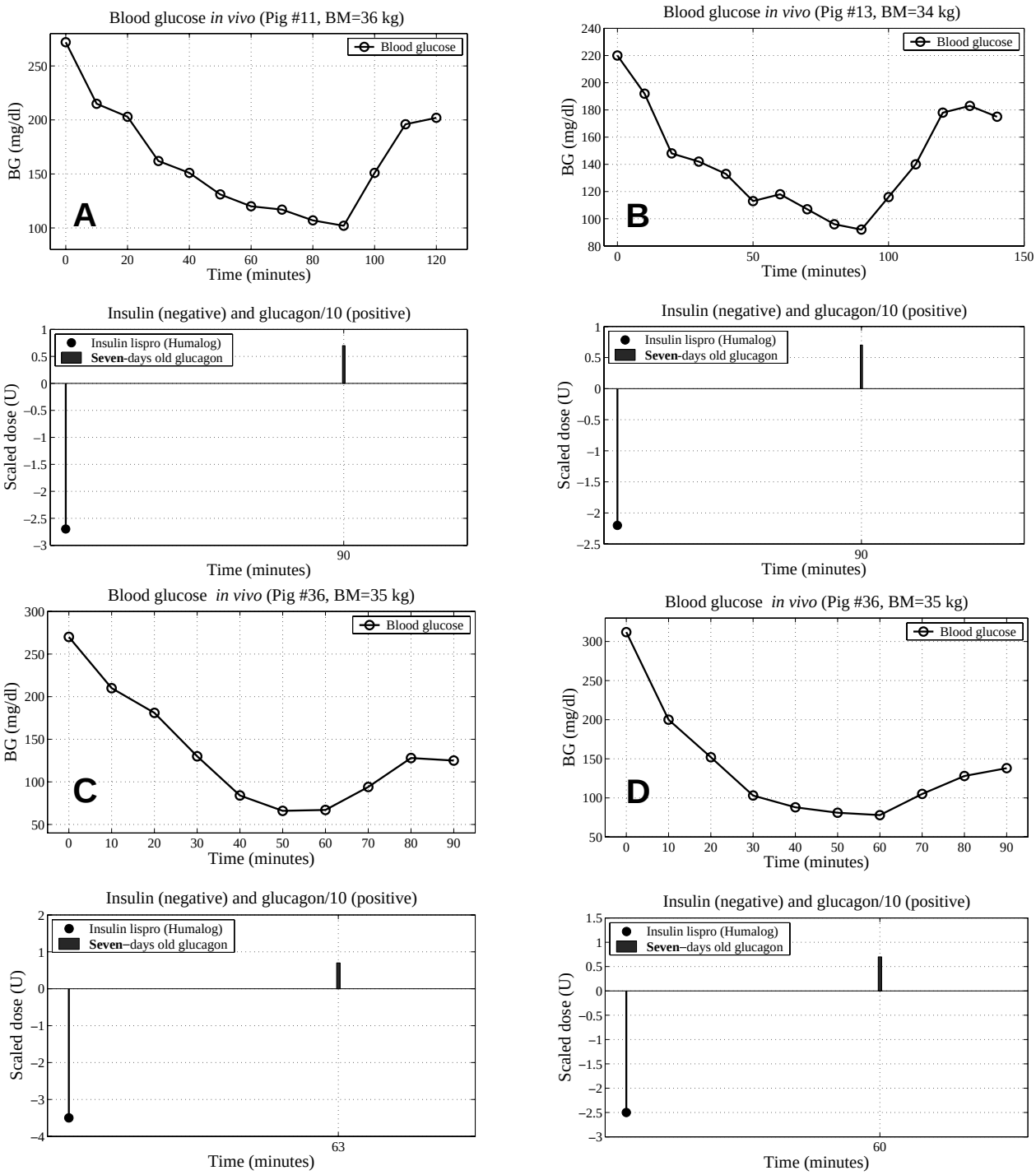


**FIG. 3.** Same explanation as in Figure 1. Notice the doubling in BG in ~20 min after glucagon administration, a phenomenon that is observed for both 7-day-old and same-day glucagon preparations (see Fig. 1).

Note the rapid glucagon action in Figures 1–4, evident by its ability to effectively halt the rapidly declining BG trend and induce a pronounced positive BG excursion. Results consistently showed that the peak effect of glucagon on BG occurred at between 10 and 30 min, but typically 20 min after SC administration, with a mean percentage increase (mean absolute increase) in BG of 102% (69 mg/dL), 64% (36 mg/dL), 215% (74 mg/dL), 139% (64 mg/dL), and 123% (90 mg/dL) for same-day and 1-day-, 3-day-, 5-day-, and 7-day-old

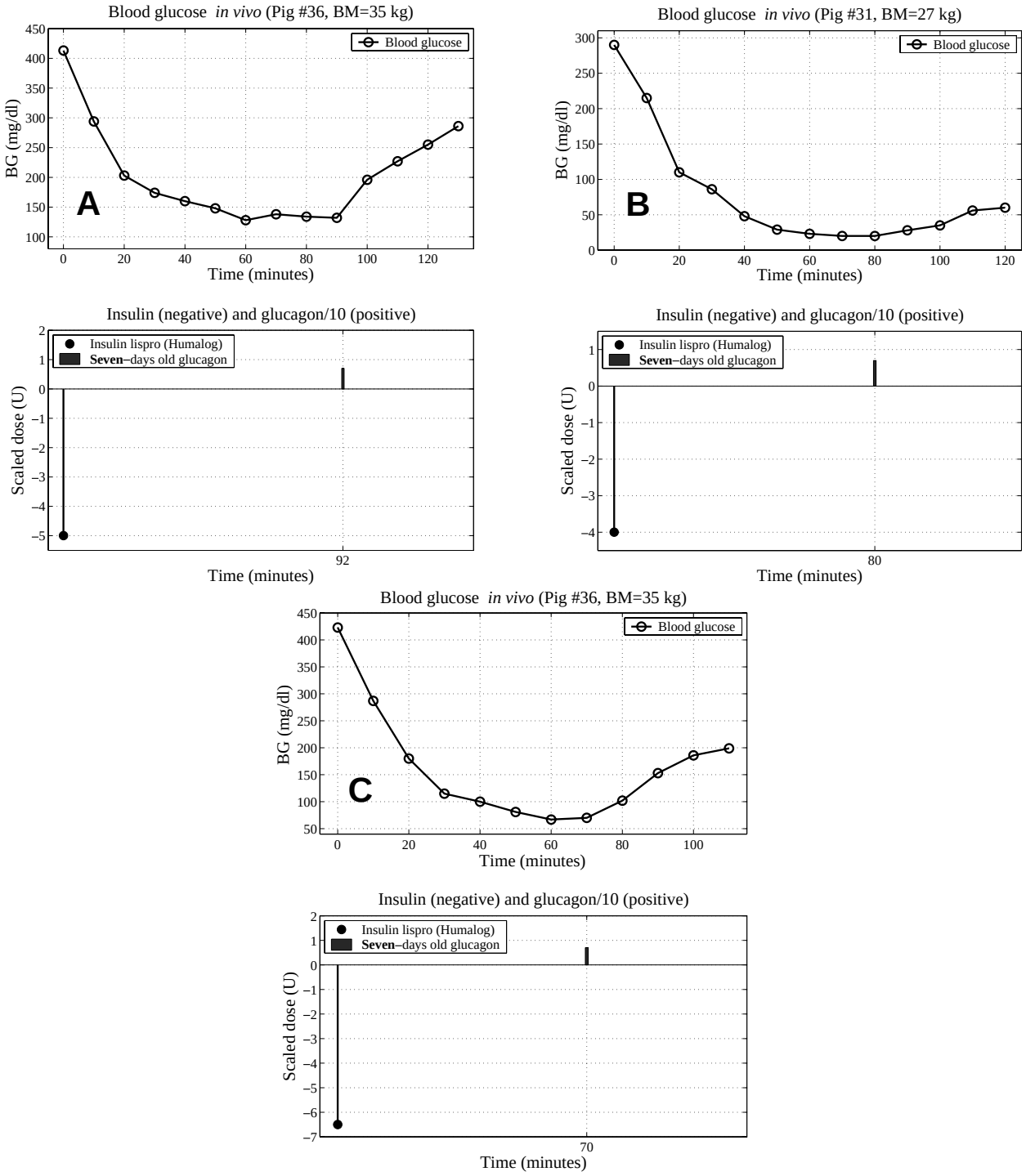


**FIG. 4.** Same explanation as in Figure 1.

glucagon solutions, respectively. For all 15 experiments shown in Figures 1–4, the mean percentage increase and mean absolute increase in BG was 127% and 74 mg/dL, respectively. The qualitative and quantitative aspects of the potency of glucagon in effecting this rebound in BG are outlined and discussed below.

## DISCUSSION

This study reveals (1) that SC dosing of glucagon that is manufactured and intended for humans is remarkably effective in our swine model of diabetes (this observation is consistent with the fact that glucagon is ho-

mologous among the vast majority of species), (2) that SC glucagon has a markedly faster absorption and effect on BG relative to insulin (as early as 5 min following SC administration), evident by its prompt halting and reversal of a declining trend in BG (for a total duration of action of about 20–30 min), (3) that a dose of glucagon that is 10 times that of insulin is roughly needed to cause a comparable but opposite change in BG, and (4) that glucagon shows no noticeable sign of depreciation or degeneration while maintained in solution at room temperature or near body temperature for up to 7 days. Note that the consistency of these results across different pigs strongly suggests that the pharmacodynamics inferred here are inherent to glucagon itself, with minimal confounding effects due to intersubject variability.

With regard to glucagon's stability, it is informative to compare results at the two extremes of our study. In particular, results of our trials with same-day glucagon (Fig. 1A and B), as with 7-day-old glucagon (Figs. 3 and 4), showed that glucagon resulted in a twofold increase in BG ~20–30 min after SC administration, with a 30–50% increase occurring in the first 10 min. In terms of absolute BG values, 7-day-old glucagon resulted in an increase of 70–170 mg/dL, whereas same-day glucagon resulted in an increase of 60–80 mg/dL, despite the smaller doses of the former (7.5 U) relative to the latter (10–20 U). [While in most of our experiments, glucagon doses were limited to between 5 and 10 U, in three experiments, larger glucagon doses were considered (between 20 and 60 U). These three experiments (Figs. 1B and D and 2D) were designed to test whether there was a threshold effect on the potency of SC glucagon. Our results suggest that the effect of glucagon on BG for doses >10 U is independent of the size of the dose. Since the pigs were fasted for about 20 h prior to anesthesia, their glycogen stores were limited, which is perhaps why no greater effect on BG was observed with doses >10 U.] Certainly these results suggest that a dose of 7-day-old glucagon stored in solution at room temperature or near body temperature shows no diminution in effect relative to a comparable dose of freshly constituted glucagon.

These results also demonstrate the efficacy of small doses (<10 U) of subcutaneously administered glucagon [typically, glucagon is administered intramuscularly only in relatively large doses (~100 U) as a rescue measure during severe hypoglycemic episodes and seizures] in rebounding a declining BG, which is consistent with a recent study that tested the effect of similar SC doses of glucagon in children with type 1 diabetes.[2] In that study, small SC doses of reconstituted glucagon demonstrated therapeutic utility in staving off impending hypoglycemia due to gastroenteritis or poor oral intake of carbohydrates. Doses between 20 $\mu$g and 150 $\mu$g (2–15 U or 0.02–0.15 mL in a standard solution concentration of 1 mg/mL) were administered to children ranging between 2 and 18 years of age, and resulted in approximately twofold increases in their BG. Results in the older adolescents also suggested extrapolation to adults, implying that "mini-doses" of glucagon offer promising utility in closed-loop control of type 1 diabetes.

The current standard of care for type 1 diabetes utilizes conventional insulin therapy that includes multiple daily insulin injections or SC insulin administration by an external infusion pump. One popular research front concerns bypassing direct persistent patient intervention by developing a closed-loop control system that would automate BG regulation.[5] The vast majority of existing or contemplated systems rely solely on insulin, while most others use dextrose or glucose as a counter agent to insulin,[6,7] and very few considered glucagon,[8,9] perhaps because of an exaggerated concern regarding its pharmacodynamics and stability in solution, a concern that is essentially annulled by the results of this study. Introducing glucagon in closed-loop control in particular, as opposed to dextrose or fast-acting sugars, uniquely mimics the physiological system in glucose regulation, namely, by accessing and utilizing the body's own glucose reserves, rather than introducing additional (exogenous) glucose. In terms of its specific role, glucagon can be administered exogenously as a counter-regulatory agent with reverse effect to that of insulin, so that the dual agents can counter hypo- and hyperglycemia, respectively. Employing glucagon can therefore substantially

increase the potential and reliability of closed-loop glucose-control systems. Its usage is further motivated by evidence that endogenous glucagon secretion is somewhat compromised in type 1 diabetes, since the (absent) beta-cells are also themselves sensors for BG concentration.[10] As a compelling attestation to its utility, the use of subcutaneously administered insulin and glucagon as dual counter-regulatory agents has recently demonstrated remarkable utility in closed-loop glucose control in an anesthetized diabetic swine model of diabetes in vivo.[3]

Although glucagon in solution is known to be much less stable than insulin at room temperature, all that is required for closed-loop glucose control is that glucagon be stable to provide an effective counter-regulatory response over a period of at most 5 days. This is because just as insulin infusion sets and insulin reservoirs are typically changed every 2–3 days during conventional pump usage, so too would glucagon infusion sets and glucagon reservoirs be changed with this frequency. Since the results of this study show no noticeable depreciation in the potency of glucagon in solution while kept at room temperature or near body temperature for as long as 7 days, we have demonstrated that there are no stability issues or outstanding concerns for employing glucagon in a closed-loop system for glucose control. Thus, with a new (disposable) cartridge filled with fresh solution every time such pump maintenance is performed, any long-term degenerative effects of glucagon or insulin are inconsequential. Furthermore, it is also possible that the stability of glucagon extends well beyond the time frame visited in this particular study, since no evidence suggesting glucagon deterioration after 7 days was observed. In either case, the stability finding of this study alleviates any concerns suspecting an inadequately short life span of glucagon in the context of closed-loop glucose control, a prevailing notion that has thus far denied glucagon such a role, when in fact it evidently has important utility in improving glucose regulation and providing safer operation[3] than could be expected from a closed-loop control system that relies on insulin alone. This is especially true given, as we have shown here, that SC glucagon is absorbed much faster and acts much faster than SC insulin. Glucagon being the faster of the two drugs in terms of bioavailability from the SC depot is a serendipitous finding that provides a rather favorable scenario for automated control of BG in type 1 diabetes, since glucagon plays a vital "safeguarding" role that would enable a closed-loop control system to eradicate impending or episodic hypoglycemia, and at the same permit using relatively aggressive insulin dosing that would minimize the exposure and duration to hyperglycemia.[3] Furthermore, note that the fact that a larger quantity of glucagon relative to insulin is needed for a comparable but opposite effect in BG will not be problematic in closed-loop employment, since glucagon is used as a sentry measure to counter impending hypoglycemia, which can only arise following an initial IV insulin overdosing. (Disturbances that affect an individual's BG level, such as carbohydrate loads or metabolic fluctuations, naturally lead to a rise in BG, rendering insulin more frequently administered and glucagon only occasionally administered in closed-loop BG control.) Thus, its more infrequent usage in closed-loop operation might offset this and result in similar consumption of both drugs over time.

## CONCLUSIONS

The potent BG effect of glucagon, which can effectively stave off impending hypoglycemia, as well as its fast in vivo absorption from the SC tissue into the bloodstream, strongly suggest profound utility in employing glucagon in closed-loop glucose control. This is further corroborated by a comprehensive in vivo study in an anesthetized swine model of type 1 diabetes, where SC doses of insulin and glucagon demonstrated robust, reliable, and safe BG regulation, with the two hormones acting as dual counter-regulatory agents.[3] Through such a system, one can see immediate utility for small doses of glucagon in a critical-care setting, with an obvious application in patients who have diabetes, and additionally in providing tight perioperative glycemic control that would clamp BG at moderate-to-low euglycemic levels in patients without diabetes, an application that has been suggested to expedite postoperative re-

covery.[11,12] Finally, given glucagon's consistent and virtually non-degrading effect while in solution at ambient conditions over a period of 7 days, its usage in the context of a closed-loop glucose-control system has promising prospects for long-term ambulatory care.

## ACKNOWLEDGMENTS

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