

## A Feasibility Study of Bihormonal Closed-Loop Blood Glucose Control Using Dual Subcutaneous Infusion of Insulin and Glucagon in Ambulatory Diabetic Swine

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### Abstract

#### Background:

We sought to test the feasibility and efficacy of bihormonal closed-loop blood glucose (BG) control that utilizes subcutaneous (SC) infusion of insulin and glucagon, a model-predictive control algorithm for determining insulin dosing, and a proportional-derivative control algorithm for determining glucagon dosing.

#### Methods:

Thirteen closed-loop experiments (~7–27 h in length) were conducted in six ambulatory diabetic pigs weighing 26–50 kg. In all experiments, venous BG was sampled through a central line in the vena cava. Efficacy was evaluated in terms of the controller's ability to regulate BG in response to large meal disturbances (~5 g of carbohydrate per kilogram of body mass per meal) based only on regular frequent venous BG sampling and requiring only the subject's weight for initialization.

#### Results:

Closed-loop results demonstrated successful BG regulation to normoglycemic range, with average insulin-to-carbohydrate ratios between ~1:20 and 1:40 U/g. The total insulin bolus doses averaged ~6 U for a meal containing ~6 g per kilogram body mass. Mean BG values in two 24 h experiments were ~142 and ~155 mg/dl, with the total daily dose (TDD) of insulin being ~0.8–1.0 U per kilogram of body mass and the TDD of glucagon being ~0.02–0.05 mg. Results also affirmed the efficacy of SC doses of glucagon in staving off episodic hypoglycemia.

#### Conclusions:

We demonstrate the feasibility of bihormonal closed-loop BG regulation using a control system that employs SC infusion of insulin and glucagon as governed by an algorithm that reacts only to BG without any feed-forward information regarding carbohydrate consumption or physical activity. As such, this study can reasonably be regarded as the first practical implementation of an artificial endocrine pancreas that has a hormonally derived counterregulatory capability.

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**Abbreviations:** (A1C) hemoglobin A1c, (BG) blood glucose, (CGM) continuous glucose monitoring, (FDA) Food and Drug Administration, (GPC) generalized predictive control, (ISF) interstitial fluid, (IV) intravenous, (SC) subcutaneous, (STZ) streptozotocin, (TDD) total daily dose

**Keywords:** counterregulatory hormone, hyperglycemia, hypoglycemia, infusion pump, *in vivo*, pig, predictive control

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## Introduction

The core technologies that will ultimately embody an automated insulin delivery system for regulating blood glucose (BG) in type 1 diabetes have been sought after for decades. However, it was not until the advent of portable continuous glucose monitoring (CGM) devices over the last decade that such a system could be practically considered. These core technologies include a drug infusion system, a CGM device, and a control algorithm.<sup>1,2</sup> Ideally, these technologies would be integrated into a safe, reliable, portable closed-loop control system that would replace conventional insulin therapies. The goal of such a system would be to improve upon the standard of care by automating glycemic control, improving hemoglobin A1c (A1C) levels, and minimizing hypoglycemia, which would all essentially work to lessen the complications of diabetes.<sup>3,4</sup>

The most likely implementation of an extracorporeal closed-loop system for outpatient usage would necessitate subcutaneous (SC) drug infusion, which, unlike intravenous (IV) administration, presents a control challenge owing to the delayed and attenuated absorption of the infused drug into the blood stream. The finite absorption rate of rapid-acting insulin analogs (with peak effect on BG typically occurring ~45–60 min after SC administration and total effect lasting ~6 h) presents the possibility of excessive drug accumulation in the SC tissue and possible hypoglycemia.<sup>5</sup> A measure that we employ to minimize or prevent this is to incorporate into the mathematical formulation of the control algorithm an optimization procedure that keeps track of and acts in light of the SC accumulation of insulin, as governed by the *in vivo* pharmacokinetics of SC insulin infusion.<sup>6</sup>

Whereas most closed-loop systems intended for outpatient usage have thus far been limited to SC infusion of insulin only,<sup>7–19</sup> a closed-loop system that would better emulate the function of the endocrine pancreas is one that employs SC infusion of both insulin and glucagon. Unlike dextrose or other fast-acting sugars, exogenous glucagon effectively mimics<sup>20</sup> and compensates<sup>21</sup> for a physiologic process in which liver glycogen stores are utilized to raise BG and, by contrast, does not introduce exogenous glucose. In past studies, we used a diabetic swine model to show that glucagon remained stable in solution for up to 7 days at room temperature or near body temperature and showed significant effect in raising BG when administered in small SC doses during closed-loop control in anesthetized diabetic pigs.<sup>6,22</sup>

These preliminary results suggest a practical role for SC infusion of glucagon in the context of an outpatient closed-loop control system. Thus the two control strategies that we exploit to avert hypoglycemia are to mathematically minimize the SC accumulation of insulin and to use glucagon as the counterregulatory agent in a bihormonal closed-loop system.

First-generation closed-loop control systems for outpatient usage will likely employ one of several commercially available technologies for sampling interstitial fluid (ISF) glucose levels. These devices have improved significantly in recent years and may have achieved a level of accuracy and reliability that is sufficient to provide the input signal for a closed-loop control system. All of the commercially available CGM devices approved by the Food and Drug Administration (FDA) estimate ISF glucose enzymatically via a transcutaneous electrochemical sensor, and all exhibit a characteristic phase lag and an attenuation in the local maxima relative to capillary and venous BG.<sup>5</sup> Such dynamics must therefore be accounted for in any control strategy that is to use an ISF glucose input signal to regulate venous BG. The rationale for first conducting closed-loop experiments based on venous BG rather than ISF glucose, as we do here, is to establish the feasibility of such a system and to isolate the control algorithm from confounding factors associated with the disparity between BG and ISF glucose.<sup>5</sup> Furthermore, a BG-based control system can be utilized whenever monitors that measure glucose from a blood vessel (e.g., noninvasive optical monitors) become available. Upon qualifying the control algorithm based on a reliable BG signal, better informed closed-loop studies can be designed in the future that operate based on an ISF-glucose input signal.

Here we present results from a bihormonal closed-loop control system that administers SC doses of insulin and glucagon in unfettered ambulatory diabetic swine under normal grazing conditions. Note that our previous study employed the same bihormonal system in relatively shorter experiments in anesthetized diabetic swine,<sup>6</sup> in which BG excursions were induced by dextrose administered intravenously via an ear vein catheter. The present study represents the first practical test of what can reasonably be regarded as the first artificial endocrine pancreas in the sense of having a hormonally derived counterregulatory capability. It is also unique in that it has been designed to deliver insulin and glucagon through a SC administration route, which makes it

readily adaptable to outpatient usage when integrated with a continuous glucose monitor. Evaluation of the performance of the closed-loop system was analyzed in terms of total daily insulin and glucagon usage, insulin-to-carbohydrate ratios delivered, and the average, maximum, and minimum BG attained.

## Materials and Methods

Experiments were conducted in unfettered ambulatory diabetic swine under normal grazing conditions, where the controller responded to venous BG measurements obtained from a central line at either 5 or 10 min intervals. Results are presented from closed-loop experiments ( $n = 13$ ) lasting between ~7 h (regulation of a hyperglycemic state followed by one meal) and ~27 h (regulation of a hyperglycemic state followed by three meals, with 4–5 h between the first and second meals and 10–11 h between the second and third meals). In two experiments, BG was regulated to the normoglycemic range for a pig (~30–80 mg/dl in venous blood) for the sake of comparison with endogenous regulation in healthy pigs; otherwise, BG was regulated to the normoglycemic range for a human (~60–120 mg/dl in venous blood). All experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Boston University (#AN-14568).

### Diabetic Swine Model

In light of similarities between pig and human in terms of skin, lipid content, SC tissue structure, and metabolism,<sup>23,24</sup> a diabetic swine model was used in all our experiments. Healthy Yorkshire swine were treated with  $\beta$ -cell cytotoxin streptozotocin (STZ) once per day for three consecutive days. Our STZ-treated swine resembled type 1 diabetes pathophysiology,<sup>6</sup> including insulin deficiency and an inability to regulate BG postprandially.

### Central-Line Catheter Insertion

To facilitate an automated method for frequent, reliable, pain-free BG sampling while the pigs are ambulatory in their runs, a central line was surgically inserted in healthy male Yorkshire swine weighing ~25 kg. The proximal end of the catheter was inserted into the jugular vein and advanced into the superior vena cava, while the distal end of the catheter was tunneled through the fascia around the neck and exteriorized at the back of the neck. The central line was used primarily to draw blood samples for reliable and accurate measurement of venous BG but also to administer IV doses of STZ for inducing diabetes.

### Induction of Type 1 Diabetes in Swine

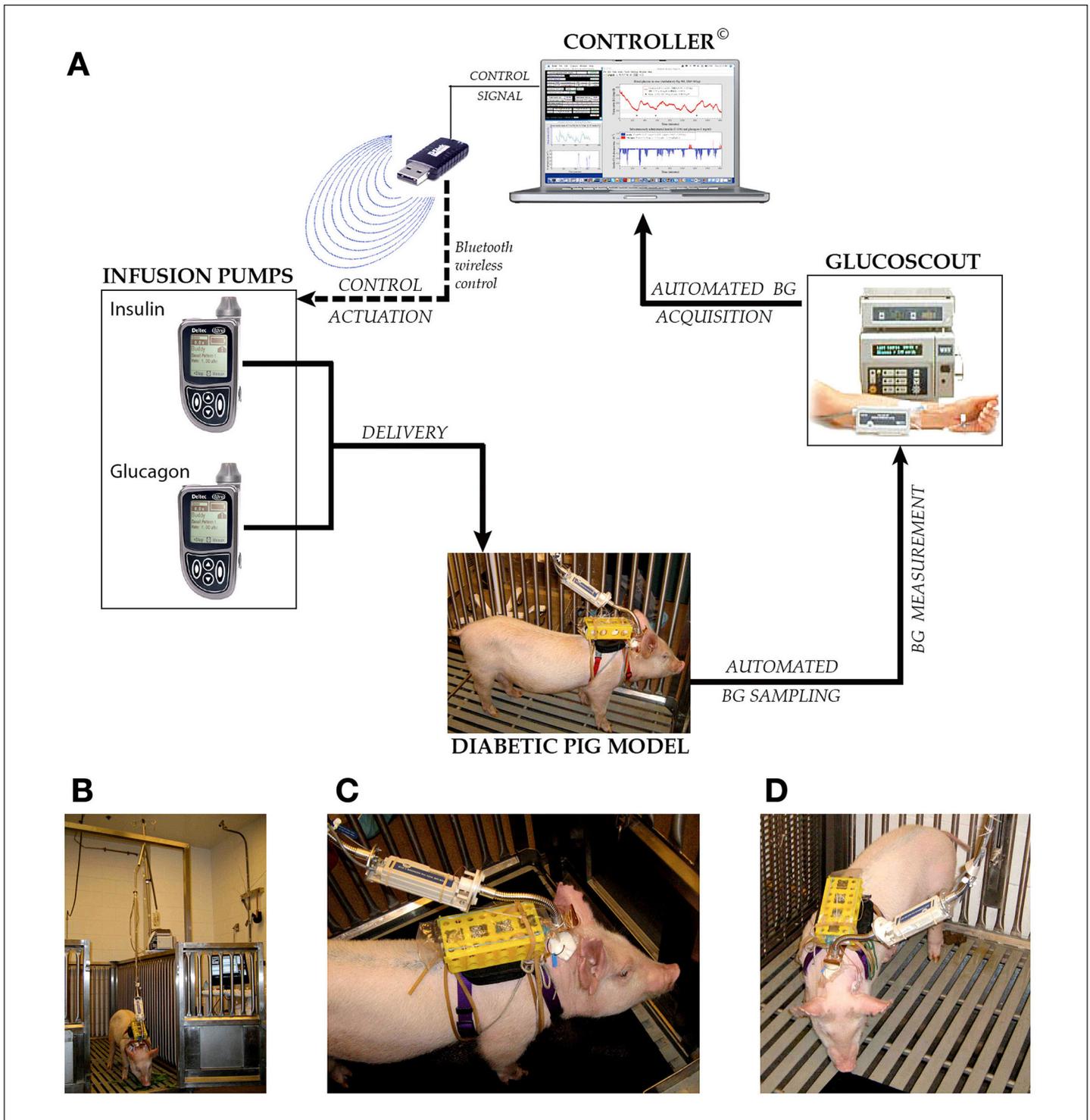
A type 1 diabetes-like pathology was induced using  $\beta$ -cell cytotoxin STZ. The STZ was mixed in 10 ml of a sodium citrate buffer solution per gram of STZ (50–80 mg/kg STZ), with pH adjusted to 4.5 using glacial acetic acid, and administered intravenously to each pig (0.5 ml of solution per 1 kg body weight) once per day for three consecutive days. Injections were administered through the central line while the pigs were awake in their stalls. The injection process was pain-free, and no restraint was necessary. Pigs were ready for experiments to commence about two weeks after injection with STZ.

### Drug Infusion and Blood Glucose Monitoring

Standard SC infusion sets (Quick-set, MMT-393, 6 mm cannula, Medtronic MiniMed) for insulin and glucagon delivery were typically placed on the pig's back. Insulin lispro (U-100, Humalog, Eli Lilly) and freshly reconstituted glucagon (Eli Lilly) were delivered through the infusion sets using customized Bluetooth-enabled Deltec CoZmo infusion pumps (Smiths Medical MD, Inc.). The total pump cartridge volume was 3 ml; however, the volume of an individual bolus rarely exceeded 10  $\mu$ l (corresponding to 1.0 U of U-100 insulin). Individual boluses were delivered in less than a minute by the pumps, which were retrofitted with Bluetooth adapters that were hardwired onto their internal serial (RS-232) ports. This customization allowed wireless pump actuation by a computer and enabled remote bolusing of insulin and glucagon doses down to the finest mechanical resolution of the device (50 nL, i.e., 0.005 U of U-100 insulin). Venous blood samples were drawn as needed via a central line, and venous BG was measured using an *in vitro* glucose meter (One Touch Ultra, Johnson & Johnson) in experiments with 10 min sampling or using a bedside glucose monitor (GlucoScout, International Biomedical, Inc.) in experiments with 5 min sampling.

### Control System

**Figure 1A** shows a paradigm illustrating the setup of our closed-loop control system. The system components include an automatic FDA-approved bedside BG monitor (GlucoScout, International Biomedical, Inc.) that was connected to the pig's central line, a computer (Mac mini or PowerMac G4, Apple, Inc.) that ran the control algorithm, and two customized Bluetooth-enabled infusion pumps (Deltec CoZmo, Smiths Medical MD, Inc.) that were secured in a modified test tube rack worn on the pig's back and used to deliver SC doses of insulin and glucagon. With each new BG value in the data stream provided by the GlucoScout, control doses of insulin



**Figure 1.** A paradigm (A) illustrating the setup of our closed-loop control system. The system components include an automatic FDA-approved bedside BG monitor (GlucoScout, International Biomedical, Inc.) that drew blood directly from the pig’s central line, a computer that ran the control algorithm and a software script that automatically streamed BG values into the computer through a serial port connection from the GlucoScout, and two infusion pumps (Deltec CoZmo, Smiths Medical MD, Inc.) that were secured in a modified test tube rack on the pig’s back and used to deliver SC doses of insulin and glucagon. Note that the infusion pumps were customized to be actuated by wireless Bluetooth signals from the control algorithm, which also allowed access to the finest mechanical resolution offered by these pumps (i.e., 50 nl) and thereby minimized round-off errors when delivering individual doses. An ambulatory pig (B) is shown inside the run while connected to the control system during a closed-loop experiment. During BG sampling, blood can be seen (C) in the central line near where the catheter exits on the back of the neck. Two infusion sets can be seen (D) inserted on the back of the neck, which are infused by two infusion pumps that are housed in the (yellow) modified test tube rack fixed on the pig’s back. Note that the pig’s central line was connected to the GlucoScout’s sensor (white and blue rectangular pack) via an articulating (white) swivel joint (visible in C and D proximal to the sensor), which was secured with tethers to allow the pig maximum mobility inside the run.

and glucagon were determined by the control algorithm, which, in turn, instructed the computer to wirelessly issue dosing commands via a Bluetooth protocol. **Figures 1B–1D** show various perspectives on an ambulatory pig inside the run during a closed-loop experiment.

### Control Algorithm

A generalized predictive control (GPC) algorithm was used to govern the SC administration of insulin [input signal  $u(t)$ ], whereas a proportional-derivative scheme was used to govern the SC administration of glucagon, both administered for the purpose of regulating BG [output signal  $y(t)$ ]. The insulin control algorithm optimizes the quadratic cost function:

$$J_{\text{GPC}} = \sum_{k=N_d}^{N_m} \delta_k \|C(r_{t+k} - y_{t+k})\|^2 + \sum_{k=0}^{N_u} \lambda_k (\Delta u_{t+k})^2, \quad (1)$$

where  $N_d$  and  $N_m$  are, respectively, the minimum and maximum (output) prediction costing horizon limits,  $N_u$  is the control horizon bound,  $\delta_k$  is the weighting on prediction error,  $\lambda_k$  is the weighting on control signals, and the integrator  $\Delta = 1 - z^{-1}$ , with  $z^{-1}$  playing the role of a one-step delay operator.<sup>25,26</sup> In order to customize the algorithm to the BG regulation problem, we augmented the GPC objective function to mathematically optimize (i) regulating BG close to a set point target within the normoglycemic range (e.g., 100 mg/dl), (ii) limiting the aggressiveness and extent of insulin administration, and (iii) minimizing the SC accumulation of insulin.<sup>6</sup> The control algorithm was initialized with only the subject's body mass and required no online information other than regularly sampled BG (e.g., no information was provided to the algorithm on the timing and carbohydrate content of meals or subject activity). The subject model used in conjunction with the GPC algorithm is a low-order empirical input–output model as we previously described.<sup>6</sup> Furthermore, hard constraints are imposed on the insulin control signal such that computed insulin doses that exceed a maximum allowable value, which is proportional to the body mass, are clipped at that maximum value.

While an augmented GPC algorithm is used to govern insulin doses, a standard proportional-derivative algorithm is used for governing glucagon doses. This is mainly due to the fact that the BG response to glucagon doses is faster than that due to insulin doses and is, in fact, comparable to the sampling frequency, as evidenced in past studies<sup>20,22</sup> and further verified in the present study. This suggests that, unlike the case with insulin, it is not critical for the glucagon algorithm to optimize the glucagon dosing based on accumulation from previous

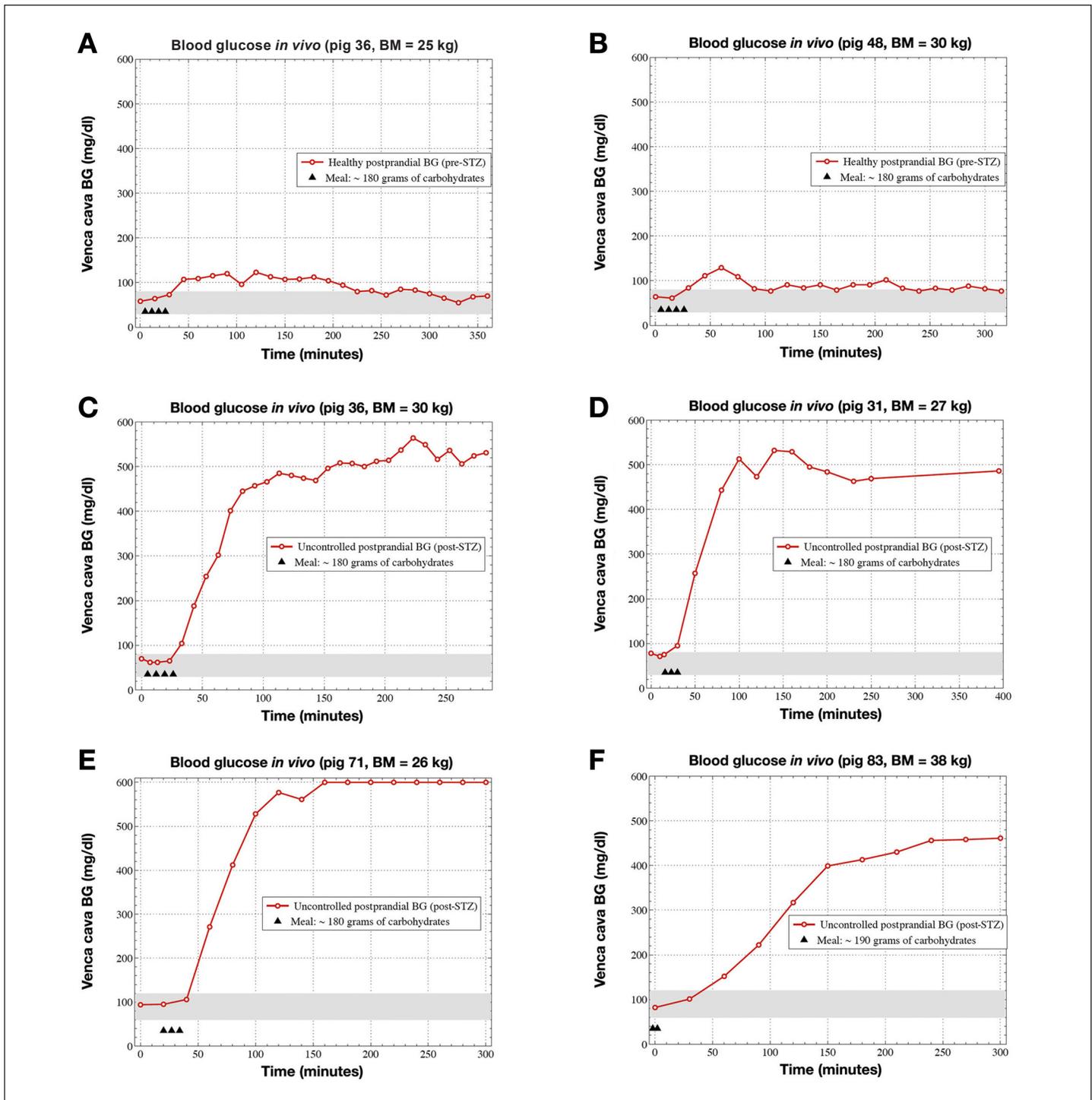
glucagon doses since the duration of action of glucagon doses is relatively brief and since glucagon serves to raise, rather than lower, BG. The two gains in the proportional-derivative algorithm for glucagon were set based on observations of the effects of microdoses of glucagon on BG in our earlier experiments in diabetic pigs,<sup>6,22</sup> as well as on observations from pediatric studies.<sup>20</sup>

## Results

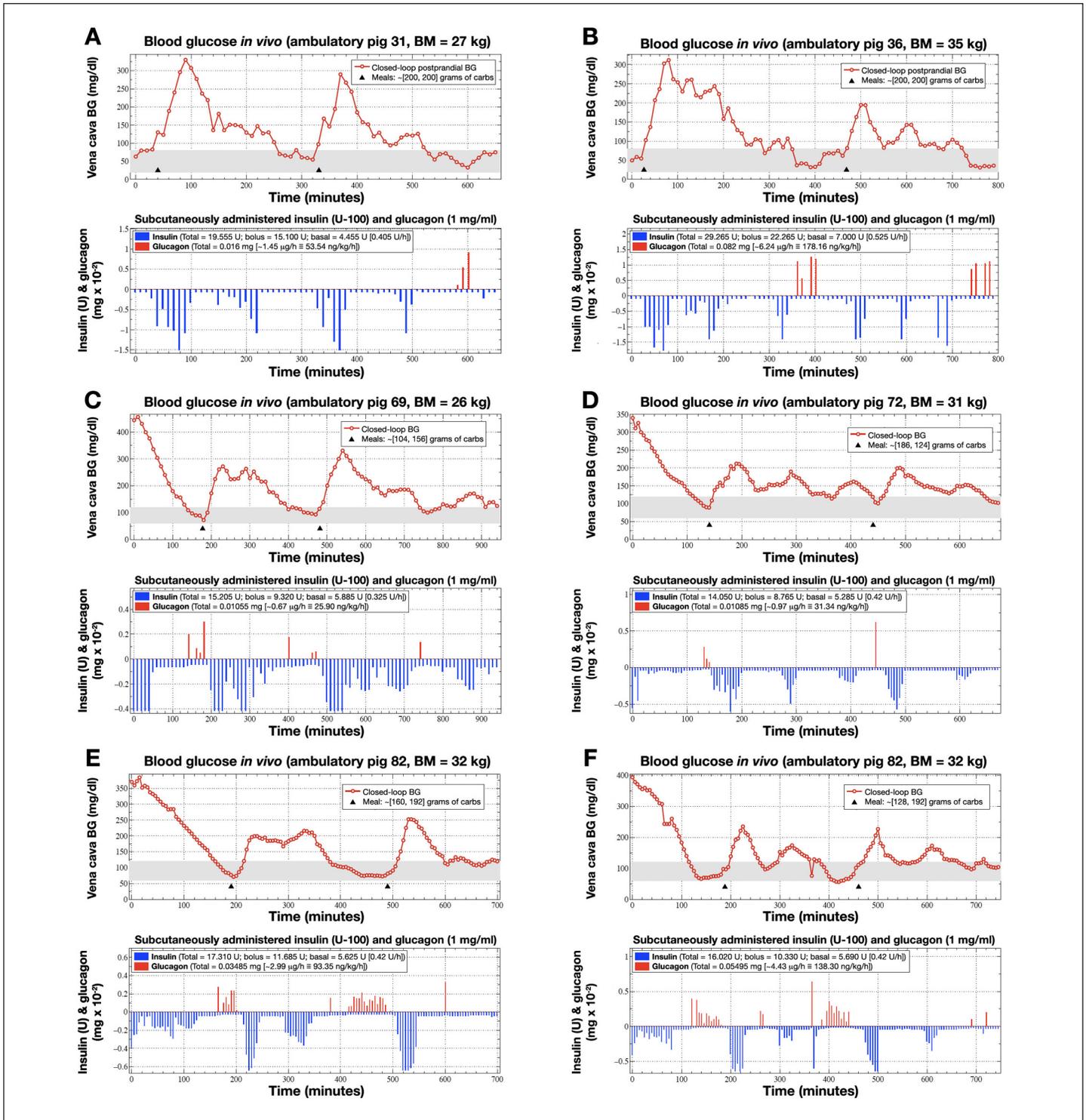
Two pre-STZ negative-control experiments (**Figures 2A** and **2B**) and four post-STZ positive-control experiments (**Figures 2C–2F**) were performed by feeding each pig a breakfast meal that contained ~180 g of carbohydrates (~5–7 g of carbohydrates per kilogram of body mass). The inability of the STZ-treated diabetic pigs to self-regulate their glucose levels is shown in **Figure 2**. Six 11–15 h closed-loop control experiments (**Figure 3**), each containing two meals, were conducted in five diabetic pigs weighing between 26–35 kg. At least one meal had a carbohydrate load of ~5–7 g of carbohydrates per kilogram of body mass (thereby providing direct comparison with the negative- and positive-control experiments). Additionally, two ~24 h closed-loop control experiments in two diabetic pigs were performed (**Figures 4** and **5**), whereby each involved three meals that, in total, contained 15 g of carbohydrates per kilogram of body mass. In these experiments, total carbohydrate consumption was divided over lunch, dinner, and breakfast so that the three meals contained, respectively, 6, 4, and 5 g of carbohydrates per kilogram of body mass in one experiment and 4, 6, and 5 g of carbohydrates per kilogram of body mass in the other. In both cases, the lunch and dinner meals were ~5 h apart, and the dinner and breakfast meals were ~10 h apart. **Table 1** provides a quantitative summary of the closed-loop experiments in **Figures 3–5**. Finally, results from five relatively short experiments in three diabetic pigs are provided in the Appendix, whereby four experiments (**Figures A1A–A1D**) included feeding the pig a single meal (each containing a carbohydrate load of ~4–6 g of carbohydrates per kilogram of body mass) and one experiment (**Figure A1E**) consisted of a small snack of pure fast-acting (cake frosting) carbohydrates (~1 g of carbohydrates per kilogram of body mass).

## Discussion

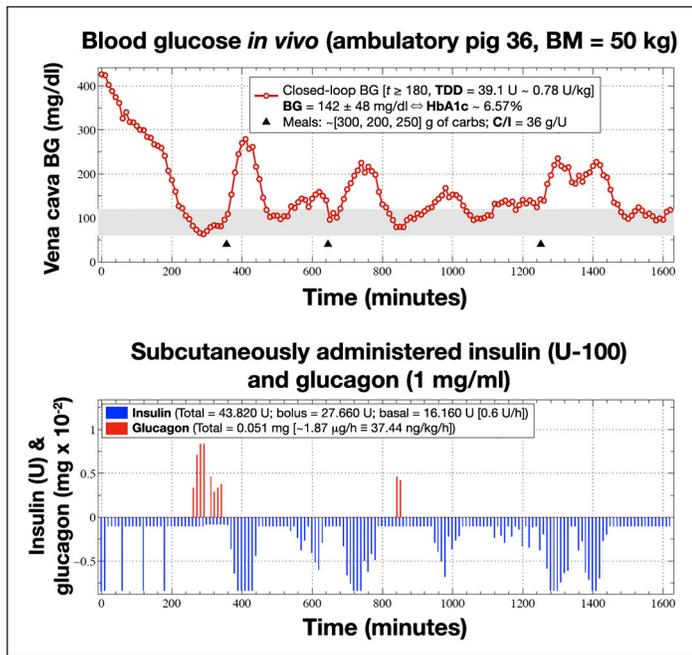
There are several results that help to assess the safety and efficacy of our control algorithm and bihormonal system to automatically regulate BG. These include the magnitude and temporal distribution of insulin dosing, the efficacy of glucagon action, the achieved mean BG,



**Figure 2.** Results from two negative-control experiments (**A** and **B**) showing endogenous postprandial BG regulation as a function of time after a breakfast meal in two healthy (nondiabetic) pigs. Note the limited extent and duration of the BG excursion (BG never exceeded 140 mg/dl) from the normoglycemic range for a pig (30–80 mg/dl in venous blood), indicated by the shaded region. Beyond an initial mild hyperglycemic excursion, BG exhibited erratic fluctuations within or near the normoglycemic range. It is also noteworthy that the postprandial BG response to lunch or dinner meals in healthy pigs (not shown here) rarely exhibited any BG departure from the normoglycemic range. Results from three positive-control experiments (**C–F**) show postprandial dysregulation of BG in four diabetic pigs. In pigs 36 and 31 (**C** and **D**), BG was reduced with IV insulin to initial values within the normoglycemic range for a pig in order to provide contrast with endogenous control in healthy pigs as well as with the results of the closed-loop experiments in **Figures 3A** and **3B**. In pigs 71 and 83 (**E** and **F**), BG was initially reduced to within the normoglycemic range for a human (60–120 mg/dl in venous blood), indicated by the shaded region, in order to provide a reference positive control for results of all closed-loop control experiments other than those of **Figure 3A** and **3B**. All negative- and positive-control experiments commenced with virtually constant BG, with essentially no outstanding effect from the initial IV insulin bolus. All BG measurements were regularly obtained from vena cava blood sampling. A BG value of 600 mg/dl was used whenever the glucometer indicated a high BG, i.e.,  $BG \geq 600$  mg/dl, which is outside the glucometer’s range. BM, body mass.



**Figure 3.** Upper subpanels in each panel show BG results from six closed-loop control experiments in ambulatory diabetic pigs, while lower subpanels show insulin–glucagon doses, which were automatically determined in real time by the governing control algorithm (scales differ between panels). Experiments involved automatically regulating two meals, at least one of which contained a carbohydrate load of ~6 g per kilogram of body mass, which is comparable to that used in the negative- and positive-control experiments shown in Figure 2. In **A** and **B**, BG was initially reduced to, and subsequently automatically regulated to, the normoglycemic range for a pig (30–80 mg/dl in venous blood), indicated by the shaded region, whereas in **C–F**, the pigs were initially hyperglycemic, and the controller automatically regulated BG to the normoglycemic range for a human (60–120 mg/dl in venous blood), indicated by the shaded region. Note that the initial hyperglycemic states in **C–F** were severe but not excessively high due to a continual subsistence level of open-loop insulin therapy being provided to the pigs prior to each experiment. In **E**, the control algorithm used successive glucagon doses to prevent a potentially severe hypoglycemic episode in the interval of ~410–430 min, a situation that had resulted from an apparent sensor BG sampling error at ~365 min that triggered a potentially excessive insulin dose. Note that ordinate scales vary. BM, body mass.

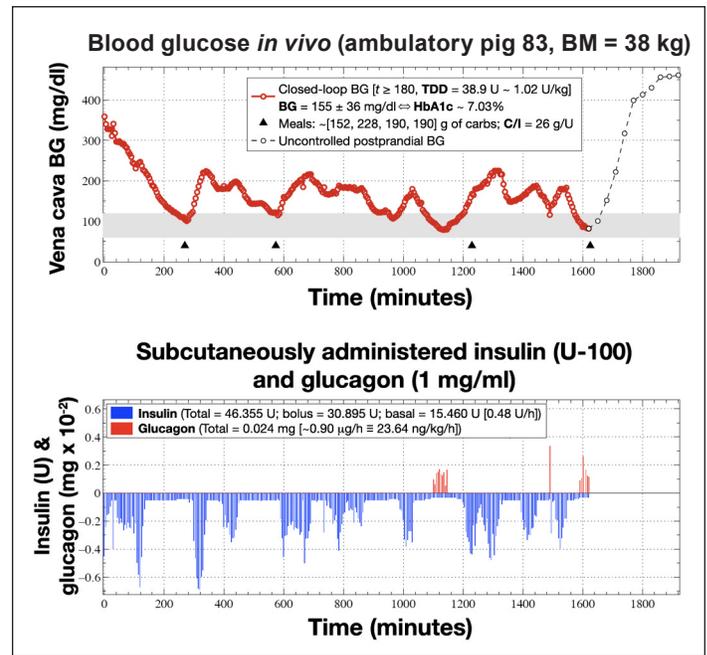


**Figure 4.** Top panel shows BG results from a closed-loop control experiment in an ambulatory diabetic pig, which lasted ~27 h and of which 24 h followed an initial severe hyperglycemic state that was regulated to a mild hyperglycemic state at ~180 min. During the course of the experiment, the pig was fed lunch, dinner, and breakfast (300, 200, and 250 g of carbohydrates, respectively), corresponding, respectively, to 6, 4, and 5 g of carbohydrates per kilogram of body mass. Note that the pig was asleep during most of the period between dinner and breakfast, with the excursion and heightened insulin requirement at ~920–1040 min possibly being due to growth hormone secretion. The control system achieved an average BG of  $142 \pm 48$  mg/dl (corresponding to a mean “virtual” A1C of ~6.57%) with no incidences of hypoglycemia. A TDD of ~39 U of insulin was administered by the controller in the 50 kg pig, with an average insulin-to-carbohydrate ratio (based on bolus doses, excluding the basal insulin component, within 5 h of all meals) of ~1.36 U/g. Furthermore, the total amount of glucagon delivered was only ~0.05 mg over a 24 h period (which is only 5–10% of the dose used clinically). The bottom panel shows the corresponding SC doses of insulin and glucagon, which were governed and determined in real time by the control algorithm.

and the frequency, extent, and duration of hyperglycemia and hypoglycemia.

### Adequacy of the Diabetic Pig Model

In order to place bounds on what is achievable with our system, given the inherent time delay associated with SC insulin infusion, and a control system that reacts only to BG (with no feed-forward information about meals), it is instructive first to consider the best- and worst-case scenarios in the form of negative-control experiments (i.e., the endogenously regulated meal response of BG in a healthy pig) and positive-control experiments (i.e., the dysregulated BG response to meals in an STZ-treated pig with no insulin therapy to treat the meal).



**Figure 5.** Same interpretation as in Figure 4. The pig was fed lunch, dinner, and breakfast (152, 228, and 190 g of carbohydrates, respectively), corresponding, respectively, to 4, 6, and 5 g of carbohydrates per kilogram of body mass. Note that the pig was asleep for most of the period between dinner and breakfast, with an excursion and heightened insulin requirement at ~960–1070 min, as observed in a similar period of sleep in the experiment of Figure 4. The control system achieved an average BG of  $155 \pm 36$  mg/dl (corresponding to a mean “virtual” A1C of ~7.03%) with no incidences of hypoglycemia. A TDD of ~39 U of insulin was administered by the controller in the 38 kg pig, with an average insulin-to-carbohydrate ratio (based on bolus doses, excluding the basal insulin component, within 5 h of all meals) of ~1.26 U/g. Furthermore, the total amount of glucagon delivered was only ~0.024 mg over a 24 h period (which is less than 5% of the dose used clinically). At the end of the ~27 h experiment, the pig was fed a meal that matched the last meal (breakfast) and the controller was switched off, thereby reemphasizing the inability of the diabetic pig to self-regulate BG postprandially, despite benefiting from preceding insulin bolus and basal doses outstanding in the SC tissue that were administered near the end of the closed-loop segment of the experiment.

Two representative negative-control experiments (see Figures 2A and 2B) show endogenous BG responses in two healthy pigs (i.e., prior to treating the pigs with STZ to induce diabetes) to a breakfast meal (pig chow) that contained ~180 g of carbohydrates, i.e., ~6 g of carbohydrates per kilogram of body mass. Since these negative-control experiments reflect the animal’s own endogenous BG regulation, they inevitably involve regulation to the normoglycemic range for a pig (~30–80 mg/dl in venous blood). The results demonstrate that, in healthy pigs, there is only a limited postprandial BG departure, where BG rarely exceeded 130 mg/dl. Note that, in the context of this study, the control responses in healthy (pre-STZ) pigs were only intended to establish a gold standard that would enable a general comparison with post-STZ

**Table 1.**  
**Summary of Closed-Loop Experiments in Figures 3–5<sup>a</sup>**

Pig ID	BM (kg)	$T_g$ (min)	Duration (min)	BG <sub>avg</sub> ± SD <sup>b</sup> (mg/dl)	Projected <sup>c</sup> A1C (%)	BG <sub>max</sub> <sup>b</sup> (mg/dl)	BG <sub>min</sub> <sup>b</sup> (mg/dl)	TDD Insulin/mass <sup>d</sup> (U/kg)	Glucagon (mg)	Insulin-to-carb <sup>e</sup> ratio (U/g)
31	27	10	650	–	–	–	–	0.93	0.016	1 : 26
36	35	10	790	–	–	–	–	1.11	0.082	1 : 20
69	26	10	940	173 ± 62	–	331	72	0.69	0.011	1 : 38
72	31	5	670	147 ± 27	–	212	89	0.69	0.011	1 : 41
82	32	5	700	140 ± 50	–	252	71	0.68	0.035	1 : 41
82	32	5	745	124 ± 39	–	235	56	0.72	0.055	1 : 37
36	50	10	1620	142 ± 48	6.57	279	63	0.88	0.051	1 : 36
83	38	5	1620	155 ± 36	7.03	225	79	1.02	0.024	1 : 26
34 ± 8 kg	–	–	650–1620	147 ± 44	6.80	256 ± 44	72 ± 12	0.84 ± 0.17	0.036 ± 0.025	1 : 33 ± 8

<sup>a</sup>  $T_g$  denotes BG sampling and control action period, and BM is body mass

<sup>b</sup> BG<sub>avg</sub>, BG<sub>max</sub>, and BG<sub>min</sub> are not reported for experiments where BG was regulated to the normoglycemic range for a pig, i.e., 30–80 mg/dl, and are reported for all other experiments while excluding the initial hyperglycemic state, or from 3 h onward, whichever comes first.

<sup>c</sup> A1C is only projected for ~24 h experiments.

<sup>d</sup> TDD of insulin is readily available in the case of ~24 h experiments and is estimated for shorter experiments based on the mean insulin-to-carbohydrate ratio and a projected daily consumption of three meals having a total of 15 g of carbohydrates per kilogram of body weight in order to match the carbohydrate consumption in ~24 h experiments.

<sup>e</sup> Insulin-to-carbohydrate ratios were computed based on insulin bolus doses, excluding the basal insulin component, that the algorithm issued within 5 h of each meal.

closed-loop and positive-control experiments in terms of emphasizing the superiority of endogenous control in dramatically limiting postprandial BG departures from the normoglycemic range. Negative-control experiments, therefore, were not conducted in all pigs. Furthermore, the slow gastric emptying associated with pig chow and glucometer inaccuracy are likely causes of the erratic BG fluctuations near normoglycemic range for a few hours after the meal. Experiments involving similar lunch or dinner meals showed tighter endogenous BG regulation, with BG hardly departing from the normoglycemic range. The response to breakfast, therefore, shows the worst-case scenario for endogenous BG regulation.

On the other hand, when fed the same meal as the healthy pigs, STZ-treated pigs showed a rapid departure in their BG (see **Figures 2C–2F**) from the normoglycemic range to an intractable hyperglycemic state (typically in excess of ~500 mg/dl) when insulin therapy for the meal was withheld. Note that, in the positive-control experiments in diabetic pigs 36 and 31 (**Figures 2C and 2D**), BG was initially brought down to the normoglycemic range for a pig with IV insulin (where all positive-control experiments commenced about 1 h after the IV insulin dose, and thus the dose had virtually no outstanding effect) in order to provide a direct comparison with the healthy

pig's endogenous control (**Figures 2A and 2B**) as well as with the two closed-loop experiments, where the controller was set to regulate BG to the normoglycemic range for a pig (**Figures 3A and 3B**). The two positive-control experiments in diabetic pigs 71 and 83 (**Figures 2E and 2F**) involved starting BG levels that were within the normoglycemic range for a human (i.e., venous BG of ~60–120 mg/dl) in order to provide a reference for the 11 closed-loop control experiments, where the controller was set to regulate to the normoglycemic range for a human (**Figures 3C–3F, 4, 5, and A1**).

In essence, the positive-control experiments verify the inability of all four diabetic pigs to regulate postprandial BG back to normoglycemic range without exogenous insulin. Furthermore, the negative- and positive-control experiments together serve as a reference for assessing the effectiveness of the closed-loop control algorithm in regulating postprandial BG. Note that the positive-control experiment in pig 83 (**Figure 2F**) was performed immediately following a ~24 h closed-loop experiment (shown in **Figure 5**) and therefore benefited from the preceding insulin bolus and basal doses that were outstanding in the SC tissue (which may account for why the hyperglycemic state achieved in this case was somewhat less than in the others).

While the BG response in positive-control experiments and closed-loop experiments is qualitatively similar across all diabetic pigs, there exists certain quantitative variability among pigs that is noteworthy. In particular, the individual insulin requirement under closed-loop control varies somewhat between pigs. For example, the insulin-to-carbohydrate ratio ranged from ~1:20 to 1:40 U/g (mean  $1.33 \pm 8$  U/g). The range and degree of variability of this quantity is remarkably similar to that which arises in children with type 1 diabetes. As such, the diabetic swine model provides a good approximation to the varied circumstances with which the controller must ultimately cope, and as evidenced by the results obtained, this variability was well managed by the controller. Further similarities in the insulin requirements of the diabetic swine model and children with type 1 diabetes are apparent in the basal insulin dosing that is administered by the controller relative to the typical dosing in open-loop therapy in children. The mean basal insulin requirement for the diabetic pigs that were regulated under closed-loop control to human normoglycemic range was  $0.39 \pm 0.03$  U/kg/day (0.32–0.41 U/kg/day). This is quite typical of the mean open-loop basal insulin requirement among children with type 1 diabetes, which has been reported as 0.38 U/kg/day in children 1–18 years of age and 0.32, 0.33, and 0.41 U/kg/day among preschooler, prepubertal, and adolescent children, respectively.<sup>27</sup> That is, the 25–50 kg diabetic swine model has a daily basal metabolic insulin requirement that is remarkably similar to that of adolescent children of the same weight. On the other hand, a comparison between the mean bolus insulin requirement of the diabetic pigs under closed-loop control and adolescent children with type 1 diabetes receiving open-loop therapy is confounded by several factors. In particular, the pig's carbohydrate consumption per unit mass, which is required by their rapid growth rate, is more typical of a human newborn (~15 g/kg/day) than a human child (~10 g/kg/day). While this might lead to the expectation that the bolus doses of insulin in the diabetic pigs would be somewhat higher than in human adolescents, this is offset, to some extent, by our observation that insulin efficiency is somewhat lower under open-loop control than under closed-loop control. We have consistently observed this phenomenon in open-loop experiments in the diabetic swine model. In particular, the same daily basal and bolus dose of insulin in the same diabetic pig consuming the same amount and distribution of carbohydrates throughout the day produces substantially worse control when the bolus doses are administered as single or distributed square-wave boluses in open-loop mode relative to closed-loop

control (see **Figure A2** in **Appendix A**). It follows that larger bolus doses would be required under open-loop mode to achieve the same mean BG as can be achieved under closed-loop control in the same diabetic pig. This is compounded by the fact that, under open-loop therapy, a poorly controlled population, in which frequent correction boluses are administered, often results in a higher total daily dose (TDD) of insulin relative to a more tightly controlled, but otherwise equivalent, population with a lower mean BG. It is therefore difficult to quantitatively compare the mean bolus insulin requirement of two populations unless both populations have the same mean BG and consume the same amount and distribution of carbohydrates. This notwithstanding, and to the extent that these competing factors might offset each other, we observed that the mean TDD of insulin in those diabetic pigs that were regulated under closed-loop control to human normoglycemic range was  $0.78 \pm 0.14$  U/kg/day (0.69–1.02 U/kg/day), which compares with 0.79 U/kg/day in children 1–18 years of age with type 1 diabetes and 0.71, 0.73, and 0.83 U/kg/day among preschooler, prepubertal, and adolescent children, respectively.<sup>27</sup>

### **Controller Performance: Insulin Dosing and Hyperglycemia**

**Figure 3** shows six closed-loop control experiments in diabetic pigs 31, 36, 69, 72, and 82. Each experiment involved regulating BG under closed-loop over a period of ~10–15 h and included two consecutive meals (mostly pig chow, plus fruit and other human foods), with at least one having a carbohydrate content of ~6 g per kilogram body mass (which, for the purpose of comparison, was chosen to be similar to that used in negative- and positive-control experiments). On the other hand, since our control algorithm regulates BG without feed-forward information about meals, it was instructive to also include meals of varying content both within an individual experiment in a particular pig as well as across experiments in different pigs. Prescribing the same meal challenge for all experiments would bring into question the critical ability of the control algorithm to robustly cope with widely varying meal challenges. In all cases, the controller successfully regulated BG to the normoglycemic range with virtually no occurrence of hypoglycemia. It should be noted that, other than requiring initialization with the subject's weight, the online operation of our control algorithm is solely based on regularly sampled BG. No *a priori* information is required by, or was provided to, the controller regarding the subject's basal insulin requirement, TDD of insulin,

or insulin-to-carbohydrate ratios under open-loop therapy, the timing or carbohydrate content of meals, physical activity, or other feed-forward information often required by other systems.<sup>10,11,16,28</sup>

Ultimately, an automatic exogenous BG controller must be able to consistently regulate BG to within the normoglycemic range in response to meals of varying carbohydrate content, as well as to other disturbances, such as exercise or hormonal activity, without causing hypoglycemia. Our 24 h closed-loop experiments (Figures 4 and 5) in pigs 36 and 83 fulfilled this goal. Of note, the meals included substantially more carbohydrates (15 g per kilogram per day) than is typical for human consumption. The TDD of insulin was consistent in both experiments, with averages of ~0.8 U/kg (pig 36) and ~1.0 U/kg (pig 83). As in the experiments of Figure 3, the total amount of glucagon that was administered did not exceed ~0.06 mg (~5–10% of the dose used clinically).

Since the experiments shown in Figures 4 and 5 span a 24 h period and contain three meals, it is reasonable to assume that the controller should be able to indefinitely maintain a mean BG equal to that achieved over the 24 h period studied. In particular, if the mean BG achieved in the 24 h experiments performed on pig 36 and pig 83 were extrapolated to a period of ~3 months, the corresponding A1C would be expected to correspond to ~6.57% and 7.03%, respectively.<sup>29</sup>

### ***Controller Performance: Glucagon Efficacy and Hypoglycemia***

The maximum cumulative total dose of glucagon administered by the controller over the course an entire experiment never exceeded 0.1 mg (<10% of the dose used clinically) and was typically between 0.02 and 0.05 mg. Individual doses were typically between 0.002 and 0.005 mg, with maximum individual doses rarely exceeding 0.01 mg. There were no indications of nausea or distress in the pigs at any time during any experiment. Despite the small size of glucagon doses administered by the control algorithm, our results suggest that glucagon played a determining role in either reversing or arresting a decline in BG. Examples of such instances can be seen in Figure 3A at ~600–630 min, in Figure 3B at ~360–430 min and again at ~740–790 min, in Figure 3E at ~420–480 min, in Figure 3F at ~130–180 min and again at ~400–450 min, in Figure 4 at ~270–300 min and again at ~840–870 min, and in Figure 5 at ~1120–1160 min. Reversal or arrest of a declining BG typically began to occur within ~10 min after glucagon administration, and in most instances, when BG reversed, the subsequent rise in BG lasted for a

period of not more than ~30 min. In some instances, the timing of glucagon doses administered by the control algorithm are confounded by the presence of a meal disturbance. For example, in Figure 3F, the rise in BG that began at ~420 min prior to the meal at ~460 min can be attributed to the successive doses of glucagon that occurred from ~400–440 min. However, the continued rise in BG that followed the meal is due, to a decreasing extent, to the residual effect of the aforementioned glucagon doses (for up to ~15 min after the meal was provided) and, to an increasing extent, to carbohydrate absorption by the gut. This supports earlier findings that showed that, relative to fast-acting insulin analogs, SC microdoses of glucagon act quickly and are short lived.<sup>6,22</sup> This is a particularly desirable property of glucagon since its role in the context of closed-loop control is to quickly arrest and counter any BG trend toward hypoglycemia without necessitating the controller to subsequently combat a protracted hyperglycemic consequence of the counterregulatory action. Taken together with our previous results,<sup>22</sup> which showed that there was no observable deterioration in potency or efficacy of glucagon when stored in solution at room temperature or near body temperature for up to 7 days, these results support the practical utility of using glucagon as a counterregulatory agent in closed-loop control.

There was only one brief instance of mild hypoglycemia using our bihormonal closed-loop system, and that was precipitated by a sensor error. Although we used the reference quality GlucoScout monitor whenever 5 min BG sampling was performed, there were several isolated instances when blood sampling errors nevertheless apparently arose. This sensor error, which can be seen in Figure 3F at 365 min and in Figure 5 at 665 min and again at 1490 min (also in Figure A1D at 290 min), is characterized by an isolated aberrant BG reading that is substantially lower than the immediately previous and immediately subsequent BG reading. On one occasion (in Figure 5 at 665 min), this sensor error did not appear to have significantly influenced either the controller action or the BG dynamics since BG was above the normoglycemic range and rising after a meal. However, on two other occasions (in Figure 3F at 365 min and Figure 5 at 1490 min), it resulted in the controller administering a glucagon bolus, as a result of the false precipitous drop in BG registered by the GlucoScout, followed at the next time step (5 min later) by an insulin bolus, as a result of the false precipitous rise in BG registered by the GlucoScout. In Figure 5 at 1490 min, the anomaly was minor and resulted in a relatively small insulin bolus (~0.3 U in a 38 kg pig), whereas in Figure 3F

at 365 min, the anomaly was much more severe and led to a relatively large insulin bolus (~0.6 U in a 32 kg pig). In the latter instance, the insulin bolus contributed to a sustained decrease in BG between ~25–50 min after the bolus was administered, which was combated by glucagon administration by the controller over this same period. Despite the fact that the peak onset of this bolus presumably occurred between ~45 and 75 min after bolus administration, this nevertheless corresponded to when BG achieved its minimum value and began to rebound (from ~50 to 80 min after bolus administration). This is compelling evidence for the effectiveness of glucagon in abating an undesirable decline in BG in the presence of a recent large insulin bolus without requiring interruption in closed-loop control or intervention with exogenous carbohydrates. A further testament to glucagon efficacy and the responsiveness of the bihormonal control system is evident in this example by the controller's ability to reduce the basal infusion rate and, more importantly, to automatically infuse glucagon, which caused the rapidly declining venous BG to abate and then quickly return to normoglycemic range after only a very brief (~10 min) exposure to mild hypoglycemia (between 56 and 60 mg/dl). It is noteworthy that, although we used raw unfiltered BG data in all our experiments, sensor filters and system-level safeguards can be designed readily into closed-loop systems that would easily allow the control algorithm to avoid this particular sensor error from influencing control action during online operation.

## Conclusions

Overall, our results demonstrate (1) successful BG regulation across an almost two-fold variation in body weight, without any feed-forward information and with virtually no hypoglycemia; (2) successful BG regulation in response to meals of different carbohydrate content, both in terms of size and complexity; (3) insulin dosing consistent with human subjects with type 1 diabetes, in terms of both TDD and the insulin-to-carbohydrate ratio used to treat meals; and (4) efficacy of the SC dual infusion of both insulin and glucagon. The results support the feasibility of safely achieving near-normal A1C levels with a reasonable therapeutic SC dose of insulin and a bihormonal closed-loop system.

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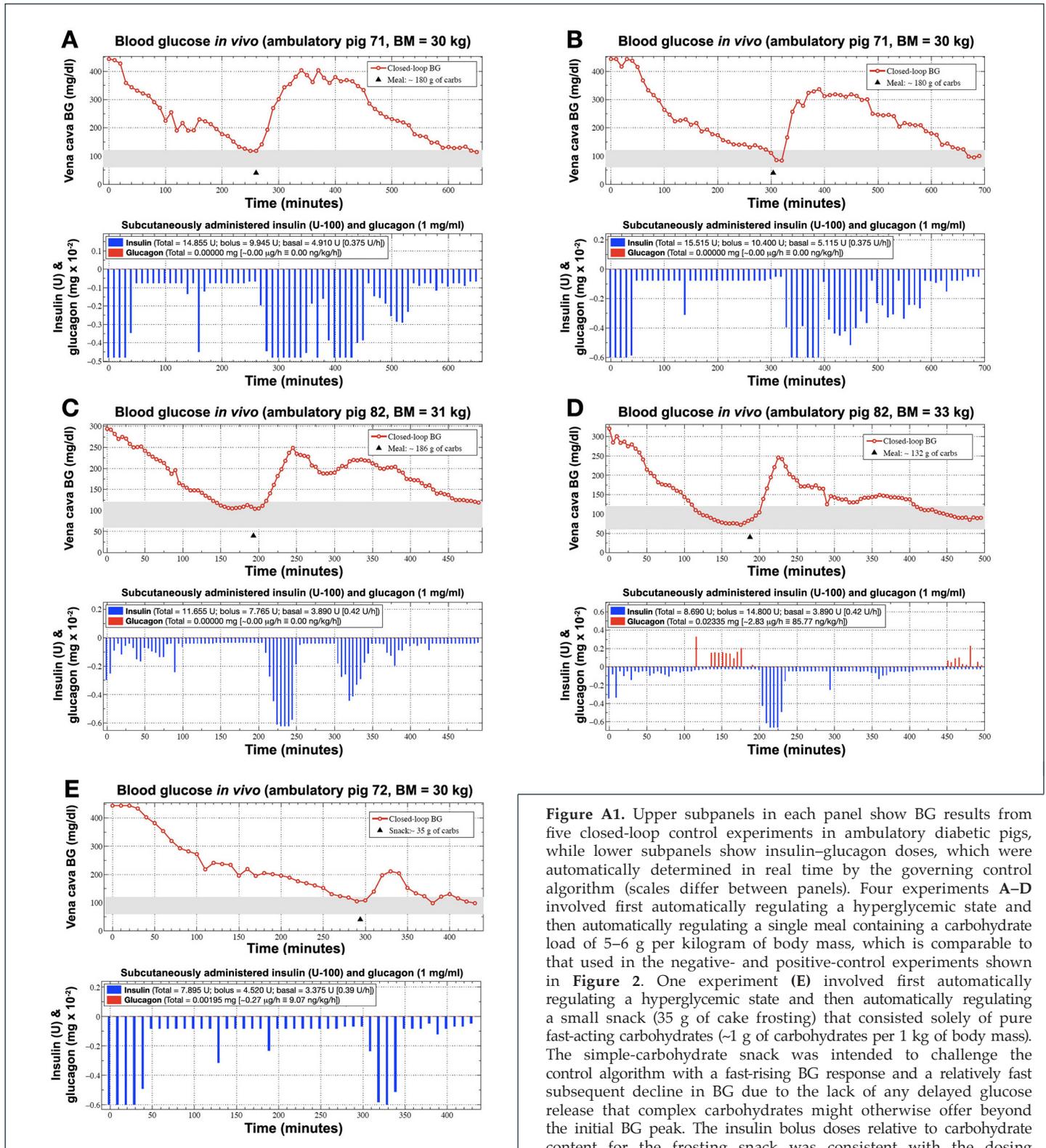
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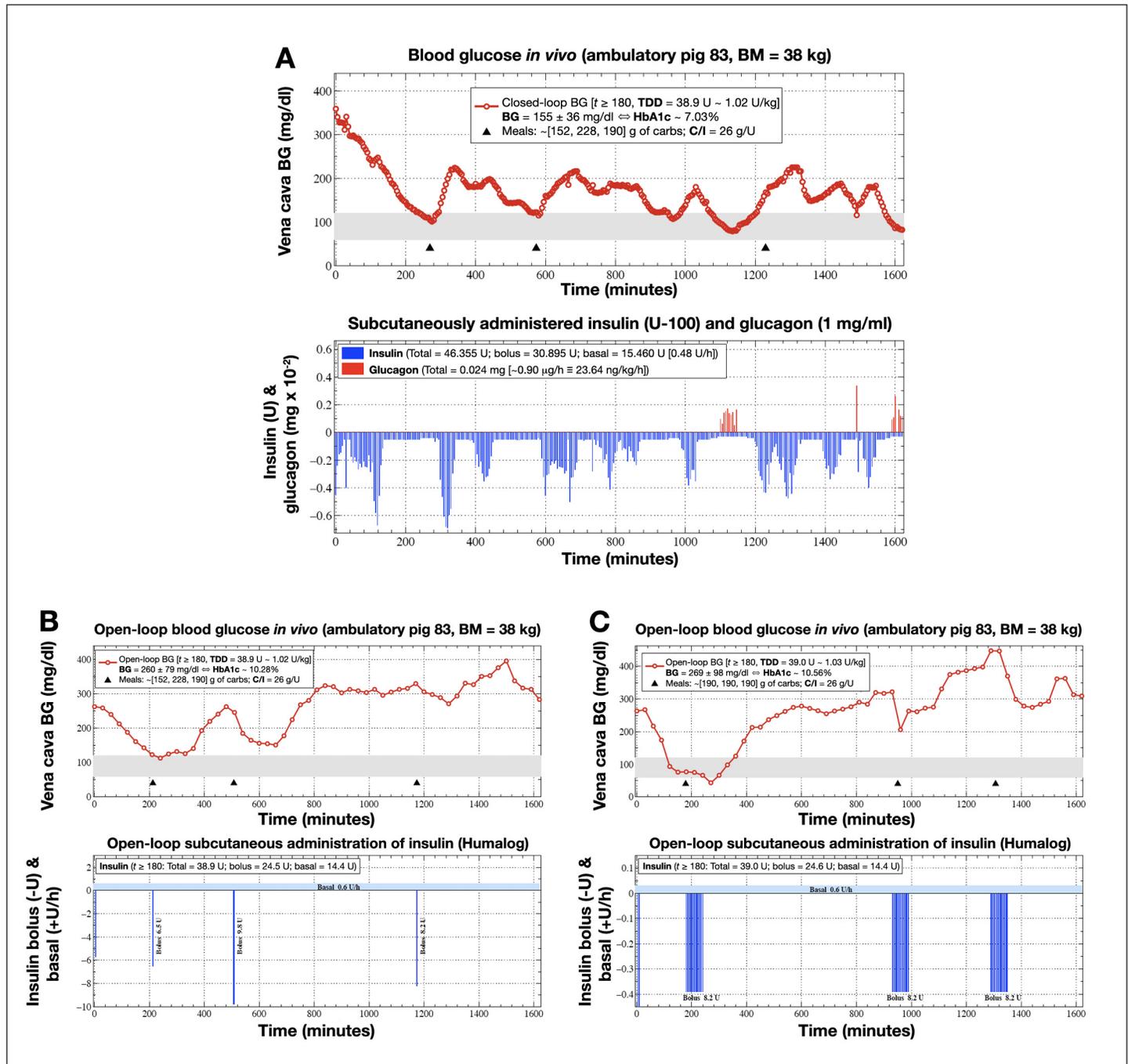
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## Appendix A: Closed-Loop Results from Single-Meal Experiments in Diabetic Swine



**Figure A1.** Upper subpanels in each panel show BG results from five closed-loop control experiments in ambulatory diabetic pigs, while lower subpanels show insulin–glucagon doses, which were automatically determined in real time by the governing control algorithm (scales differ between panels). Four experiments A–D involved first automatically regulating a hyperglycemic state and then automatically regulating a single meal containing a carbohydrate load of 5–6 g per kilogram of body mass, which is comparable to that used in the negative- and positive-control experiments shown in Figure 2. One experiment (E) involved first automatically regulating a hyperglycemic state and then automatically regulating a small snack (35 g of cake frosting) that consisted solely of pure fast-acting carbohydrates (~1 g of carbohydrates per 1 kg of body mass). The simple-carbohydrate snack was intended to challenge the control algorithm with a fast-rising BG response and a relatively fast subsequent decline in BG due to the lack of any delayed glucose release that complex carbohydrates might otherwise offer beyond the initial BG peak. The insulin bolus doses relative to carbohydrate content for the frosting snack was consistent with the dosing observed in all other closed-loop experiments involving complex carbohydrates (pig chow), with an average of ~1 U per 30 g of carbohydrates. All five experiments involved regulating BG to the normoglycemic range in a human (60–120 mg/dl), indicated by the shaded region, starting from initial hyperglycemic states that were severe but not excessively high due to a continual subsistence level of open-loop insulin therapy being provided to the pigs prior to each experiment. Note that, in all five experiments, BG was successfully regulated to normoglycemic range with no occurrences of hypoglycemia. Note that ordinate scales vary.



**Figure A2.** Panel A shows results from the closed-loop control experiment in pig 83 that are presented in Figure 5, where the control system achieved a mean BG of  $155 \pm 36$  mg/dl and administered a TDD of 38.9 U over 24 h (starting from  $t \geq 180$  min) in a 38 kg pig. The TDD was distributed by the closed-loop system as 14.4 U in basal doses (0.6 U/h, on average) and 24.5 U in bolus doses, which resulted in an overall insulin-to-carbohydrate ratio of 1:26 U/g. Panels B and C show two 24 h experiments that were conducted under open-loop mode in the same pig. In each open-loop experiment, the pig was fed three meals, which were identical to the three meals consumed under closed-loop control as shown in A, and received a basal rate of insulin of 0.6 U/h along with bolus insulin doses that were based on the carbohydrate content of each meal and the insulin-to-carbohydrate ratio of 1:26 U/g that was observed under closed-loop control. In the experiment of panel B, the bolus doses were administered as a single bolus dose per meal at the time the meal was provided, whereas, in the case of the experiment of panel C, square-wave boluses were administered over 30 min starting at the time each meal was provided. Thus the two open-loop experiments and the closed-loop experiment were all conducted in the same pig under the same carbohydrate challenge and using the same TDD of insulin. Furthermore, the distribution of the TDD of insulin between total basal and total bolus dose components was the same in all three experiments. Nevertheless, the BG regulation in both open-loop experiments was markedly inferior to that obtained under closed-loop control, with 24 h means in BG of  $\sim 260$  mg/dl under open-loop mode as opposed to 155 mg/dl under closed-loop control (in all cases, 24 h mean BG was computed for  $t \geq 180$  min after BG was near or within target range).