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DIABETES

A Bihormonal Closed-Loop Artificial Pancreas for Type 1 Diabetes

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Automated control of blood glucose (BG) concentration is a long-sought goal for type 1 diabetes therapy. We have developed a closed-loop control system that uses frequent measurements of BG concentration along with subcutaneous delivery of both the fast-acting insulin analog lispro and glucagon (to imitate normal physiology) as directed by a computer algorithm. The algorithm responded only to BG concentrations and incorporated a pharmacokinetic model for lispro. Eleven subjects with type 1 diabetes and no endogenous insulin secretion were studied in 27-hour experiments, which included three carbohydrate-rich meals. In six subjects, the closed-loop system achieved a mean BG concentration of 140 mg/dl, which is below the mean BG concentration target of ≤154 mg/dl recommended by the American Diabetes Association. There were no instances of treatment-requiring hypoglycemia. Five other subjects exhibited hypoglycemia that required treatment; however, these individuals had slower lispro absorption kinetics than the six subjects that did not become hypoglycemic. The time-to-peak plasma lispro concentrations of subjects that exhibited hypoglycemia ranged from 71 to 191 min (mean, 117 ± 48 min) versus 56 to 72 min (mean, 64 ± 6 min) in the group that did not become hypoglycemic (aggregate mean of 84 min versus 31 min longer than the algorithm's assumption of 33 min, $P = 0.07$). In an additional set of experiments, adjustment of the algorithm's pharmacokinetic parameters (time-to-peak plasma lispro concentration set to 65 min) prevented hypoglycemia in both groups while achieving an aggregate mean BG concentration of 164 mg/dl. These results demonstrate the feasibility of safe BG control by a bihormonal artificial endocrine pancreas.

INTRODUCTION

Achieving and maintaining near-normal blood glucose (BG) concentrations are critical for successful long-term care of patients with diabetes mellitus. The Diabetes Control and Complications Trial and its long-term follow-up demonstrated the importance of maintaining glycated hemoglobin (HbA1c), an index of the mean BG concentration, as close to the nondiabetic range as possible in individuals with type 1 diabetes (*1*–*3*). The internationally adopted treatment goal (*4*) of maintenance of HbA1c values at <7% (corresponding to a mean BG of ~154 mg/dl) reduces the development and progression of microvascular and cardiovascular complications by as much as 76% (*1*, *2*). Unfortunately, the therapy required to achieve this goal is extremely demanding, necessitating frequent self-monitoring of BG concentrations and multiple daily insulin injections or use of an insulin pump. Even with physiologic insulin replacement in the form of a continuous insulin delivery throughout the day combined with bolus doses of insulin at meals (basal-bolus therapy), substantial hyperglycemic excursions and episodic hypoglycemia persist in most people with type 1 diabetes (*5*–*7*). Hypoglycemia can result in life-threatening consequences and limits the application of intensive therapy. The development of a drug delivery device that responds to glucose concentrations and automatically "clamps" BG concentrations in the nondiabetic range, a so-called artificial endocrine pancreas, has been a long-term goal to avoid the negative consequences of type 1 diabetes.

Closed-loop BG control devices require a stream of frequent glucose concentration measurements for operation. The prospect for the development of such devices has been aided by recent improvements in minimally invasive continuous glucose monitoring (CGM) and by an improved understanding of the physiologic control of glycemia (*8*–*10*). In individuals without diabetes mellitus, glucose concentrations are maintained between 70 and 180 mg/dl through the interplay of insulin and glucagon secreted by the pancreatic islets (*11*). Insulin is secreted in response to elevated BG and other physiologic signals and facilitates disposal of glucose into the liver and other peripheral tissues. Glucagon counters the effects of insulin and increases glucose production by the liver, stabilizing glucose concentrations after meals and preventing hypoglycemia.

Previously reported studies testing closed-loop BG control systems using subcutaneous insulin infusion have not included a physiological counterregulatory component such as glucagon (*12*–*16*). Those studies with experiments lasting 24 hours or more reported repeated occurrences of hypoglycemia, which required intervention with carbohydrate administration, as required by their protocols (*17*, *18*).

On the basis of the physiological principles of endogenous BG regulation, we have developed a computer control algorithm that makes automated dosing decisions for subcutaneous insulin and glucagon administration based on regularly sampled BG concentrations measured every 5 min. This BG control algorithm has previously been tested in a porcine model of insulin-deficient diabetes (*17*, *18*). We report here our results with this bihormonal artificial endocrine pancreas in human subjects with type 1 diabetes.

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RESULTS

Eleven subjects with type 1 diabetes, no endogenous insulin secretion, and HbA1c values <8.5% participated in at least one closed-loop experiment. The baseline characteristics of the subjects are shown in Table 1. Each experiment included 27 hours of closed-loop BG control during which subjects consumed three standardized carbohydrate-rich meals.

Closed-loop control system

The closed-loop control system consisted of three components (fig. S1): a venous BG monitor, infusion pumps to deliver insulin and glucagon subcutaneously, and a computer-based control algorithm that automatically computed insulin and glucagon doses to be administered to the subject based on regularly sampled BG concentrations. Insulin lispro and glucagon were delivered through catheters (infusion sets) inserted into the subcutaneous tissue of the abdomen. The control algorithm was initialized only with the subject weight and used BG measurements every 5 min as the sole input. As such, the system was entirely reactive and did not benefit from a premeal insulin priming bolus (*13*) or include any meal announcement or meal prediction strategies (*14*, *15*). A customized model predictive control (MPC) algorithm [Supplementary Material (SM) Note 1] governed subcutaneous insulin lispro dosing with the goal of achieving a BG concentration of 100 mg/dl (*17*–*19*). The algorithm incorporated a pharmacokinetic (PK) model of the subcutaneous absorption and clearance from blood of lispro and took into account both model-estimated subcutaneous and plasma lispro concentrations (SM Note 1). In the initial studies, the model parameter values assigned to t_{max}, the time-to-peak plasma lispro concentration, and $t_{95\%}$, the time to 95% clearance of plasma lispro concentration, were 33 min and 3.25 hours, respectively. This t_{max} value is within the range reported by the manufacturer in the package insert for lispro (30 to 90 min) and was found to give good results in preclinical experiments in diabetic swine (*17*, *18*). A proportional-derivative (PD) control algorithm, with an online accumulation term, governed subcutaneous glucagon dosing (SM Note 2) with the goal of preventing or treating excursions of BG concentration below 100 mg/dl.

Glycemic control and drug delivery

Two patterns of BG control emerged in the 11 initial studies (Fig. 1, A and D, Table 2, and figs. S2 to S12). In six subjects (later determined to have relatively faster lispro PK), no hypoglycemia requiring intervention occurred (for example, Fig. 1A); however, carbohydrate interventions were required to treat hypoglycemia in five subjects, later determined to have relatively slower lispro PK (for example, Fig. 1B). For the six subjects requiring no intervention, the closed-loop system achieved an aggregate mean BG of 140 mg/dl, with only two episodes of asymptomatic biochemical hypoglycemia (<70 mg/dl) in the total 133 hours of closed-loop control (Fig. 1A, Table 2, and figs. S2 to S7). The lowest BG for these experiments was 66 mg/dl. Seventy-four percent of study time was spent with BG in the American Diabetes Association (ADA) glycemic target range of 70 to 180 mg/dl (*4*), and <1% of time was spent below 70 mg/dl. There was a postprandial hyperglycemic excursion after each meal. This was anticipated because the algorithm was entirely reactive and commanded insulin doses only after the BG concentration began to rise; thus, there was a delay in insulin dosing in response to meals. A delayed rise in postprandial plasma insulin levels was further compounded by the time required for absorption of subcutaneously infused insulin into blood, inevitably resulting in a period of postprandial hyperglycemia.

Most of the prandial insulin was provided in the hour after initiation of the meal (for example, Fig. 1, B and E). The control algorithm commanded additional insulin doses if the BG concentration remained above the target BG of 100 mg/dl and the algorithm estimated that plasma insulin and insulin pending in the subcutaneous depot were insufficient to regulate the BG excursion. If the slope of the fall in BG was steep as it approached the target, or if BG fell below the target, the controller commanded glucagon doses that typically resulted in a rapid change in the slope of BG (for example, Fig. 1A). Glucagon doses were small relative to the typical 1-mg dose used clinically to treat severe hypoglycemia; the largest single dose in a 5-min interval was 20 μg. The total glucagon administered ranged from 0.120 to 0.377 mg per 24 hours (mean, 3.14 μg/kg per 24 hours) in this group. Perhaps because individual and total glucagon doses delivered by the control algorithm were relatively small, there were no adverse events

Table 1. Baseline characteristics of subjects. Results are expressed as mean ± SD (range), unless otherwise stated. BMI, body mass index.

	All subjects	Subjects requiring extra carbohydrates*	Subjects not requiring extra carbohydrates*
Number	11	5	6
Sex	7 M/4 F	5 M	2 M/4 F
Age (years)	40 ± 16 (19–71)	47 ± 19 (19–71)	34 ± 13 (20–50)
Body mass (kg)	83 ± 13 (66–110)	90 ± 12 (79–110)	78 ± 11 (66–95)
BMI (kg/m^2)	28 ± 3 (22–31)	27 ± 4 (22–31)	28 ± 3 (22–31)
Diabetes duration (years)	23 ± 13 (6–46)	30 ± 17 (6–46)	17 ± 4 (9–21)
HbA1c (%)	7.3 ± 0.8 (6.2–8.5)	7.4 ± 1.0 (6.4–8.5)	7.3 ± 0.7 (6.2–8.1)
Daily insulin dose (U/kg)	0.6 ± 0.2 (0.3–1.0)	0.5 ± 0.2 (0.3–0.8)	0.7 ± 0.2 (0.5–1.0)
Stimulated C-peptide (nM)†	<0.03	<0.03	<0.03

*Hypoglycemia was treated with extra carbohydrates (15 g) if BG remained <60 mg/dl for a 20-min period, <50 mg/dl for a 10-min period, or if subjects were symptomatic. †All subjects had undetectable fasting and stimulated C-peptide, reported as less than the assay detection limit.

associated with glucagon delivery. Specifically, no subject had symptoms of nausea or gastrointestinal discomfort.

The performance of the closed-loop system and the resultant BG pattern in the initial studies of the other five subjects was markedly different (Fig. 1D, Table 2, and figs. S8 to S12). Each of these subjects developed hypoglycemia (20 events of BG <70 mg/dl in 104 hours of closed-loop control), including at least one episode requiring oral carbohydrate treatment per experiment (mean of 3.4 doses of 15 g of carbohydrates per experiment, total of 17 carbohydrate interventions). One experiment was terminated early, as per protocol, because of the need for three doses of oral carbohydrate in 1 hour and intravenous dextrose administration. The aggregate mean BG concentration was not significantly different between this group and the six subjects without hypoglycemia (144 versus 140 mg/dl), owing to more time spent in the hypoglycemic range (13% versus <1% of BG values <70 mg/dl, $P = 0.007$) and in the hyperglycemic range (36% versus 25% of BG values >180 mg/dl, $P = 0.12$). The hypoglycemic events typically occurred in the late postprandial period despite more glucagon delivery in this group (mean, 8.05 μg/kg per 24 hours versus 3.14 μg/kg per 24 hours, $P = 0.02$). The attenuation or reversal in the downward BG trend after glucagon administration noted in the group without treatment-requiring hypoglycemia was typically not evident in these subjects.

Insulin PK data

Analysis of insulin lispro PK in the 11 initial closed-loop experiments suggested that differences in the rate of lispro absorption and clearance were responsible for intersubject variability in closed-loop system performance. There was a large variation in lispro PK between subjects, with t_{max} ranging from 56 to 191 min and $t_{95\%}$ ranging from 5.6 to 19.1 hours (Table 2). The six subjects without treatment-requiring hypoglycemia exhibited an average lispro t_{max} of 64 ± 6 min (56 to 72

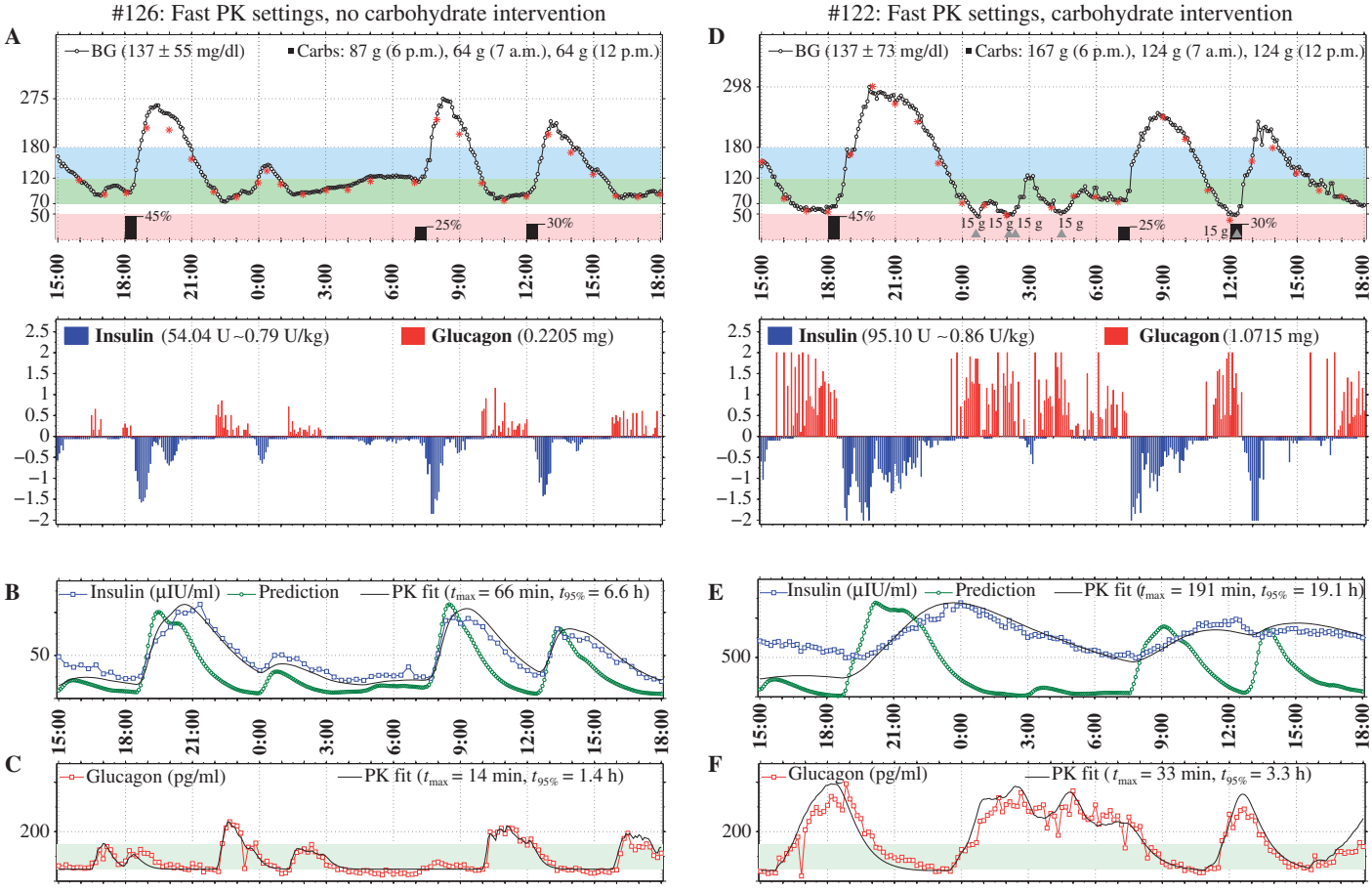

Fig. 1. Representative results from two closed-loop BG control experiments of the initial studies. (**A** to **C**) Results from a subject who did not develop treatment-requiring hypoglycemia and, in retrospect, had relatively fast insulin lispro PK. (**D** to **F**) Results from a subject who did require carbohydrate treatment and had slower lispro PK. (A and D) Venous BG concentrations measured every 5 min with GlucoScout (black circles). Gray triangles along the timeline in (D) indicate 15-g carbohydrate treatments for hypoglycemia. Hourly confirmation values (red stars) obtained with an independent glucose analyzer (YSI) are superimposed on the BG trace. Meals are indicated along the timeline by rectangles, with the percentage of daily calories in each meal indicated. Boluses of insulin (vertical blue bars with negative amplitudes) and glucagon (vertical red bars with positive amplitudes) commanded by the algorithm are shown below the BG concentrations in (A) and (D). (B and E) Model-estimated (green circles) and measured (blue squares) insulin concentrations. The black line is the best-fit trace of the biexponential lispro PK model to the measured insulin concentrations (SM Note 3). (C and F) Measured plasma glucagon concentrations (red circles). The black line is the best-fit trace of the biexponential glucagon PK model to the measured glucagon concentrations.

min). The five subjects requiring carbohydrate treatment for hypoglycemia displayed nearly double the average lispro t_{max}: 117 ± 48 min (71 to 191 min). Among the five subjects requiring carbohydrate treatment for hypoglycemia, the one with the lowest lispro t_{max} (71 min) required carbohydrate treatment only once, whereas all of the other subjects in this group had greater t_{max} values (mean, 128 min; 78 to 191 min) and required carbohydrate treatment two or more times.

A greater disparity between the measured lispro concentration profiles and the model-estimated profiles used by the control algorithm was evident in the five subjects who required carbohydrate intervention. Specifically, the aggregate mean t_{max} was 84 min versus 31 min longer than the algorithm's t_{max} parameter setting of 33 min ($P = 0.07$) for the subjects with and without treatment-requiring hypoglycemia, respectively (Fig. 1B and figs. S8 to S12). This greater disparity correlated with more postprandial hyperglycemia. When configured with the initial t_{max} parameter setting of 33 min, the algorithm could not anticipate the subsequent absorption of insulin that had accumulated in the subcutaneous depot in subjects with slower lispro PK. Consequently, the control algorithm commanded more insulin doses in response to hyperglycemia, which led to plasma insulin concentrations in the late postprandial period that were excessive and resulted in hypoglycemia refractive to microdoses of glucagon.

Glucagon PK data

Analysis of glucagon PK in the initial studies (Fig. 1, C and F, and figs. S2 to S12) revealed that glucagon was consistently absorbed more rapidly than insulin lispro across all 11 subjects (mean glucagon t_{max} of 23 ± 9 min versus mean lispro t_{max} of 90 ± 43 min, $P < 0.001$). The range of measured mean glucagon concentrations was 49 to 97 pg/ml in subjects

Table 2. Summary of closed-loop experiments. Statistics are reported for 24 hours, starting at 6 p.m. on admission day and ending at 6 p.m. on the next day, except in (three) cases where the experiment was discontinued earlier.

Controller PK setting	Subject ID #	BG_{avg} ± SD (mg/dl)	Projected HbA1c (%)*	[BG_{min}, BG_{max}] (mg/dl)	Number of carbohydrate interventions†	Inferred lispro PK		Percentage time spent				Total lispro (U/kg)	Total glucagon (μg/kg)
						t_{max} (min)	$t_{95\%}$ (hours)	≤70	70–120	70–180	>180		
Fast	108‡	139 ± 60	6.5	[73, 269]	0	63	6.3	0	62	72	28	0.66	2.89
	110	142 ± 50	6.6	[67, 264]	0	72	7.2	3	38	72	25	0.80	3.47
	117	128 ± 52	6.1	[66, 264]	0	56	5.6	2	63	78	20	0.67	4.43
	119‡	156 ± 57	7.1	[80, 267]	0	—	—	0	39	66	34	1.02	1.47
	126	137 ± 55	6.4	[74, 275]	0	66	6.6	0	51	78	22	0.79	3.21
	128	137 ± 41	6.4	[74, 229]	0	62	6.2	0	40	81	19	0.73	3.38
	Mean	140 ± 9§	6.5	[72, 261]	0	64	6.4	1	49	74	25	0.78	3.14
	115	144 ± 65	6.7	[47, 287]	1	71	7.1	6	42	61	33	0.91	5.46
	121‡	—	—	[37, 357]	3	111	11.1	14	18	28	58	—	—
	122	137 ± 73	6.4	[45, 298]	5	191	19.1	19	35	52	29	0.86	9.74
	129	164 ± 89	7.3	[45, 360]	2	132	13.2	9	37	53	38	1.17	6.71
	132	129 ± 68	6.1	[32, 269]	6	78	7.8	18	40	59	23	0.84	10.3
	Mean	144 ± 15§	6.6	[41, 314]	3.4	117	11.7	13	34	51	36	0.95	8.05
Slow	110	154 ± 51	7.0	[78, 266]	0	69	6.9	0	34	70	30	0.59	1.15
	117	161 ± 63	7.2	[78, 313]	0	68	6.8	0	34	68	32	0.69	2.93
	126	146 ± 46	6.7	[82, 254]	0	50	5.0	0	42	73	27	0.54	1.70
	128	153 ± 51	7.0	[84, 256]	0	72	7.2	0	38	64	36	0.57	1.26
	115	176 ± 57	7.7	[99, 286]	0	46	4.6	0	23	56	44	0.71	0.02
	121	179 ± 75	7.9	[64, 319]	0	80	8.0	3	26	50	47	0.77	2.75
	122	155 ± 57	7.0	[69, 264]	0	127	12.7	<1	38	65	35	0.59	2.51
	129	198 ± 72	8.5	[92, 333]	0	141	14.1	0	19	46	54	0.85	0.32
	132	157 ± 69	7.1	[76, 293]	0	50	5.0	0	46	65	35	0.68	2.67
	Mean	164 ± 17§	7.4	[80, 287]	0	78	7.8	<1	33	62	38	0.67	1.70

*Reported HbA1c values are projections based on mean BG (*24*). †Carbohydrate interventions were administered according to protocol. ‡Experiments were discontinued because of loss of intravenous access in #108 (statistics are reported for 15.75 hours) and #119 (statistics are reported for 21.33 hours) and because of intervention with intravenous dextrose in the first experiment in #121 (statistics are reported for 7.5 hours). §Mean and SD in BG across subjects were computed based on the individual mean BG values.

without treatment-requiring hypoglycemia and 62 to 195 pg/ml in subjects that did require carbohydrate treatment (glucagon normal range, 50 to 150 pg/ml).

Repeat closed-loop studies after adjusting lispro PK parameter settings

To test the hypothesis that the disparity between model-estimated and measured insulin concentrations was responsible for hypoglycemia, we adjusted the PK parameter settings of the algorithm to a lispro t_{max} of 65 min, twice the value used in the initial studies. We then performed repeat closed-loop experiments in each of the five subjects who had required carbohydrate treatment in the initial studies (Fig. 2, A to C, and figs. S13 to S17) and in four of the six subjects who did not develop treatment-requiring hypoglycemia in the initial studies (Fig. 2, D to F, and figs. S18 to S21). In the repeat experiments, closed-loop BG control was achieved without any treatment-requiring hypoglycemia, albeit with an aggregate mean BG concentration of 164 mg/dl (Table 2). Whereas the average lispro t_{max} value among the nine subjects participating in repeat experiments was not significantly different from their initial studies [78 ± 34 min (46 to 141 min) versus 93 ± 44 min (56 to 191 min), P = 0.43], model-estimated plasma insulin concentrations by the controller corresponded more closely to measured insulin concentrations in the repeat experiments than in the initial studies. Specifically, lispro t_{max} for these nine subjects was on average only 13 min longer than the new algorithm setting of 65 min in repeat experiments compared with 61 min longer than the initial algorithm setting of 33 min in the initial studies (P = 0.02). In the five subjects who developed treatment-requiring hypoglycemia in the initial studies, less glucagon was administered in their repeat experiments (1.65 μg/kg per 24 hours versus 8.05 μg/kg per 24 hours, P = 0.006). There was a smaller, albeit significant, decrease in the glucagon administered in the repeat experiments of the four subjects who did not require carbohydrate treatment in the initial studies (1.76 μg/kg per 24 hours versus 3.62 μg/kg per 24 hours, P = 0.01). Unlike in the initial studies, the total daily glucagon dose in the repeat studies was similar in these two groups (1.65 ± 1.4 μg/kg per 24 hours versus 1.76 ± 0.81 μg/kg per 24 hours, respectively, P = 0.89). The range of measured mean glucagon concentrations was 49 to 139 pg/ml (glucagon normal range, 50 to 150 pg/ml). Summary BG and plasma insulin profiles for all of the studies are shown in Fig. 3.

Cumulative BG profiles demonstrate that in the initial studies the faster PK group spent the majority of time (74% on average) in the ADA glycemic target range with no treatment-requiring hypoglycemia, whereas in the slower PK group there was both more hypoglycemia and hyperglycemia and only 51% of time was spent in the ADA target range (Fig. 4, A and C). In the repeat experiments (Fig. 4E), the distribution of BG results was compressed, with no hypoglycemia. A

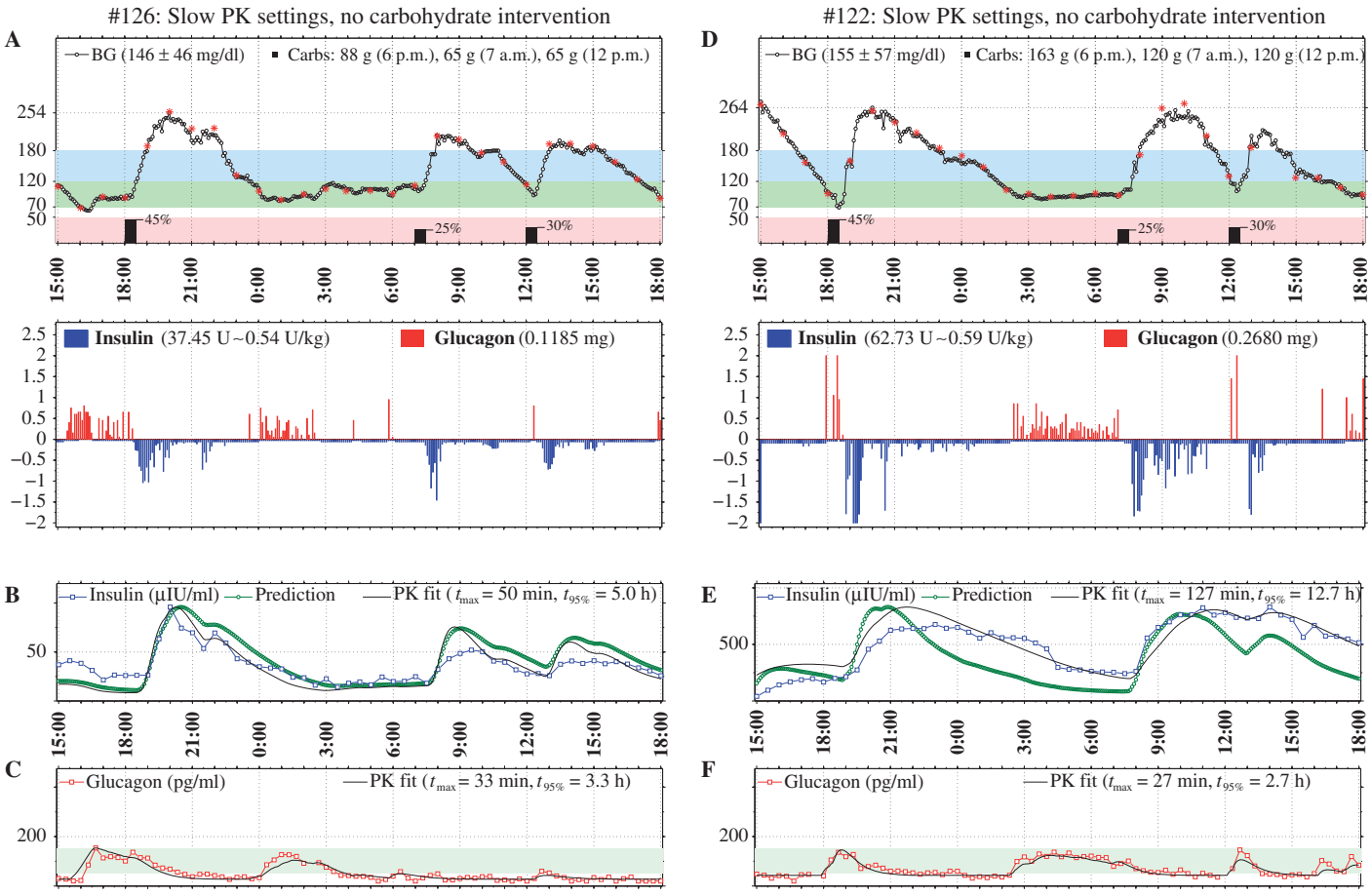

Fig. 2. (**A** to **F**) Representative results from repeat closed-loop experiments for subjects #126 and #122 (Fig. 1) are shown using the slower PK parameter settings in (A to C) and (D to F), respectively. All panels display the data in the same way as their counterparts in Fig. 1.

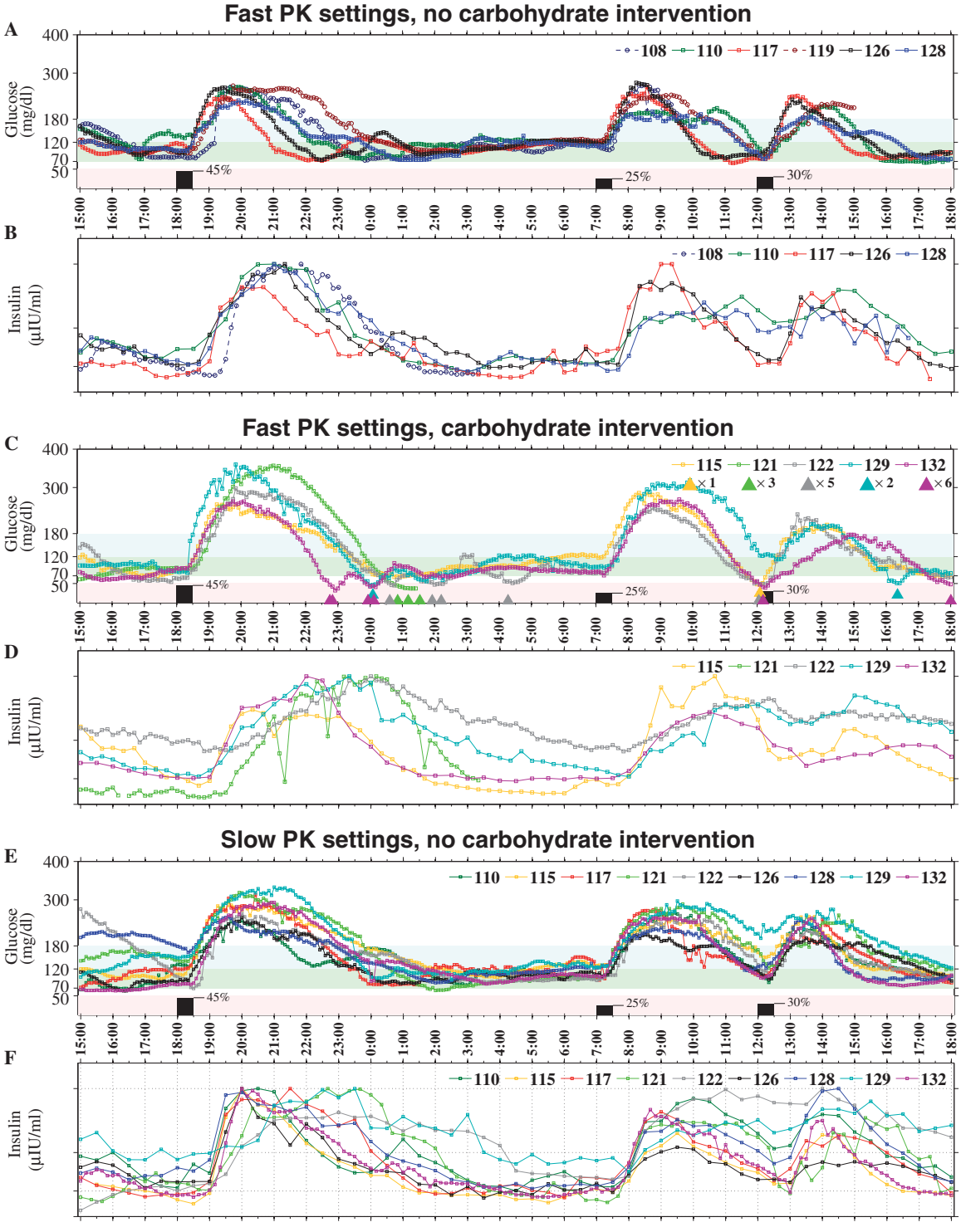

Fig. 3. Venous BG and plasma insulin concentrations from closed-loop BG control experiments in all 11 subjects. (**A** and **B**) Results in the six subjects who did not develop treatment-requiring hypoglycemia. (**C** and **D**) Results in the five subjects who required one or more carbohydrate treatments for hypoglycemia during the initial experiments using the controller configured with the original fast lispro PK parameter settings (t_{max} = 33 min). Each 15-g carbohydrate intervention for hypoglycemia is indicated along the timeline in (C) with a color-coded triangle. (**E** and **F**) Results of the repeat experiments using the controller configured with the slow PK settings (t_{max} = 65 min).

comparison was performed between the algorithm's PK settings and graphical representations of the lispro PK for each subject derived from their measured insulin concentrations (Fig. 4, B, D, and F). It is evident that when there was less disparity between the model-estimated PK and the subject's PK (compare Fig. 4, B and D), the time spent in the ADA glycemic target range was greater and there was no treatment-requiring hypoglycemia (compare Fig. 4, A and C). In the repeat experiments, model-estimated lispro PK was in closer agreement with each subject's PK (Fig. 4F), and treatment-requiring hypoglycemia was eliminated (Fig. 4E). Note that in the initial studies, the fast PK parameter settings resulted in model-estimated PK that was faster than any subject's measured PK (Fig. 4, B and D), whereas in the repeat experiments the slow PK parameter settings resulted in model-estimated PK that fell between the fastest and slowest subjects' measured PK (Fig. 4F).

In the repeat experiments, glucagon consistently attenuated, arrested, or reversed the downward slope of BG (Fig. 2 and figs. S13 to S21), consistent with the conclusion that hypoglycemia with the fast PK settings was due to excessive insulin rather than insensitivity to glucagon. The efficacy of glucagon in preventing hypoglycemia in these repeat experiments as well as in the initial experiments in subjects with faster PK is suggested by positive changes in the time derivative of BG after glucagon dosing. Whereas these positive changes are not consistent with the slow decay of lispro levels in the blood (minimum lispro $t_{95\%}$, 4.6 hours), they are consistent with the rapid absorption of glucagon (mean glucagon t_{max}, 23 ± 9 min); see, for example, the BG plots in Fig. 1A at 22:30 and 10:00, and in Fig. 2A at 16:30 and 0:30 and Fig. 2D at 2:30.

DISCUSSION

We have demonstrated the feasibility of safe BG control with a bihormonal closed-loop BG control system in individuals with type 1 diabetes. Near-normal mean BG concentrations without hypoglycemia were achieved without feedforward information or pretreatment for very high carbohydrate meals in the subjects with faster insulin PK. In subjects with slower insulin absorption, adjustment of the algorithm's PK parameters prevented hypoglycemia at the cost of modestly higher average BG concentrations. Other clinical trials testing closed-loop control with subcutaneous insulin infusion have reported multiple episodes of hypoglycemia in several subjects (*12*, *13*). Although Steil *et al.* (*12*) suggested adding an insulin feedback mechanism to their proportional-integral-derivative (PID) control algorithm to avoid excessive insulin dosing, the implementation of this approach has not been reported in a closed-loop system using subcutaneous insulin infusion. A modification of their PID algorithm to include insulin feedback has been implemented and tested in a closed-loop system in which insulin was delivered intraperitoneally with an implantable insulin pump (*20*). Despite this modification, multiple episodes of hypoglycemia requiring carbohydrate administration occurred (*20*). In contrast, we have identified discordance between measured and model-estimated insulin concentrations as the most likely cause of the hypoglycemic episodes that we observed in some subjects. We were able to eliminate hypoglycemia in these same subjects by a single modification of the PK parameters that was then applied in all repeat experiments. Our success is likely due to the fact that our algorithm accounts for the combined effect of insulin accumulation at the administration site (the subcutaneous depot) as well as in plasma. Thus, our algorithm is responsive to the instantaneous appearance of a subcutaneous insulin bolus at the infusion site as well as to the accumulation of that bolus over time in the plasma. This capability was first suggested and formally incorporated into a closed-loop BG control algorithm by El-Khatib and Damiano (*19*), and subsequently implemented in preclinical experiments in diabetic swine by El-Khatib *et al.* (*17*, *18*) and in clinical experiments in human subjects with type 1 diabetes in the present study. Our results suggest that the ability of a BG control algorithm to account for subcutaneous insulin PK, as ours does, is essential for safe and effective BG control with subcutaneous lispro infusion. In contrast, the PID algorithm with insulin feedback used by Renard *et al.* (*20*) accounted only for the accumulation of insulin in plasma and neglected accumulation at the site of administration. This formulation may have been adopted because insulin was administered intraperitoneally. Although this is likely to lead to considerably faster absorption than with subcutaneously administered insulin, insulin accumulation at the site of administration may still occur because absorption into plasma is not instantaneous.

In addition to accounting for subcutaneously infused insulin PK, a second factor that may account for the robustness that we observed in our controller is the availability of glucagon to stave off impending hypoglycemia, provided that the effect of the glucagon was not overwhelmed by a large excess of insulin. The inclusion of glucagon in our system was designed to imitate normal physiology and prevent the postprandial hypoglycemia that has been seen in closed-loop studies using only subcutaneous insulin infusion (*12*, *13*). Although each individual glucagon dose was small, glucagon administration appeared to slow, arrest, or reverse the descent in BG. The very rapid onset of glucagon counterregulatory action (mean glucagon t_{max}, 23 ± 9 min) appeared to contribute to the success of the closed-loop system in preventing hypoglycemia. When modest amounts of excess insulin had been delivered, glucagon dosing was associated with a rapid positive change in the derivative of BG with respect to time. This appeared to buy time for the insulin concentration in the blood to decay until the ambient insulin concentration was in equilibrium with BG and homeostasis was achieved. However, when the disparity between measured and model-estimated lispro concentrations was too large, and large amounts of excess insulin accumulated, the small doses of glucagon delivered by the controller were not sufficient to prevent hypoglycemia. After adjustment of the lispro PK parameters to more closely approximate lispro absorption in these individuals, glucagon apparently contributed to preventing hypoglycemia in all repeat experiments, even in subjects with slower lispro absorption. The glucagon control algorithm could be modified to provide escalating doses of glucagon if the BG response to initial glucagon doses was not adequate, thereby providing a larger margin of safety for prevention of hypoglycemia.

The BG values we achieved were generally in the nondiabetic range between meals and overnight but higher than normal after meals. The postprandial glucose excursions are a consequence of the glucose control algorithm being entirely reactive (that is, delivering insulin only in response to a rise in BG concentration) and the relatively slow absorption of subcutaneous insulin. The system therefore requires some time to catch up after a carbohydrate load. To address the postprandial hyperglycemia, a small premeal insulin "priming" bolus (to

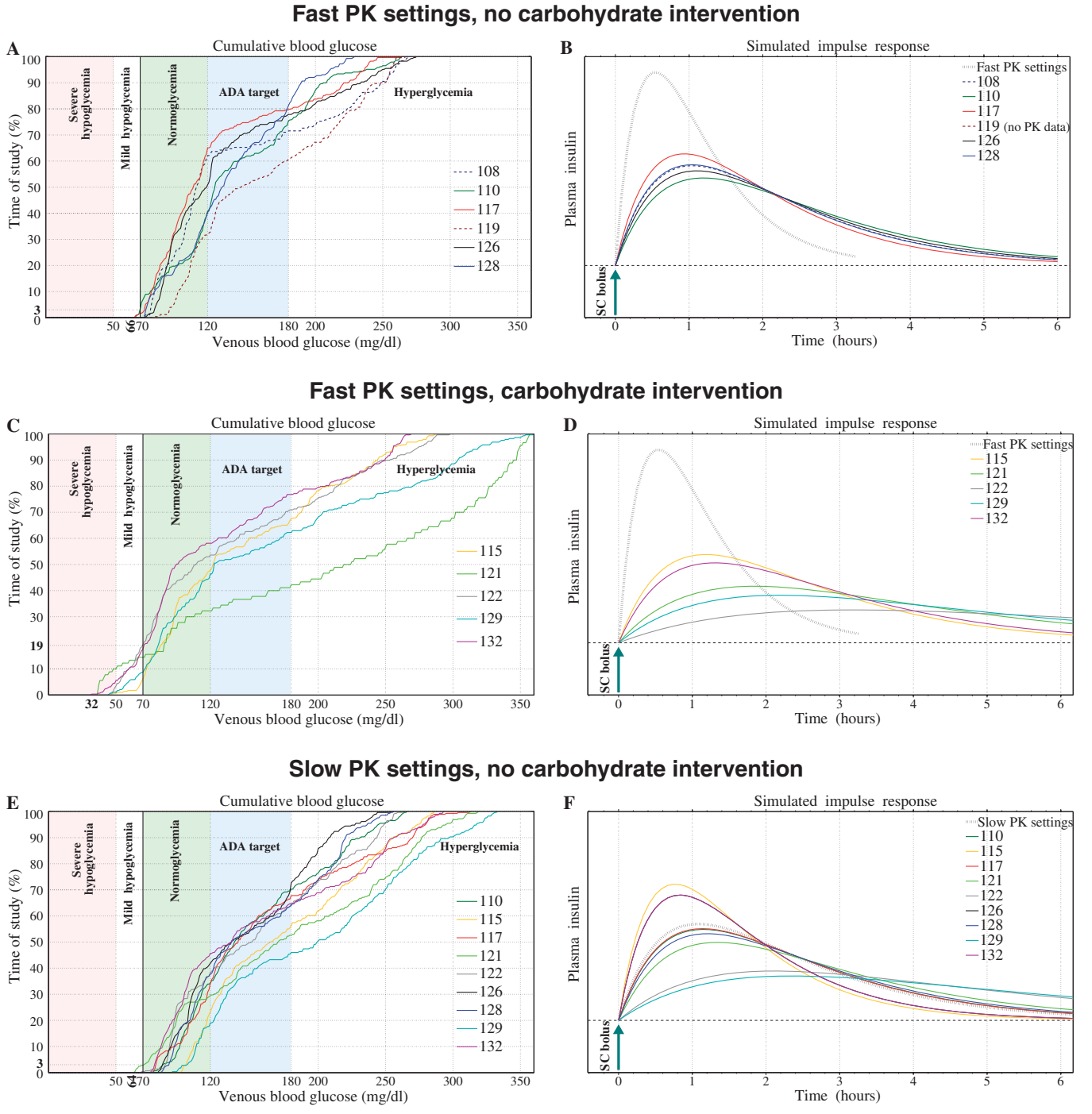

Fig. 4. Cumulative BG concentrations and lispro PK from closed-loop BG control experiments in all 11 subjects and corresponding simulated insulin profiles derived retrospectively and portrayed as the lispro concentrations after a single subcutaneous (SC) insulin bolus (SM Note 3). (**A** and **B**) Cumulative venous BG concentrations (A) and simulated insulin profiles (B) from the six subjects who did not develop treatment-requiring hypoglycemia. (**C** and **D**) Cumulative BG concentrations (C) and simulated insulin profiles (D) from the five subjects who required one or more carbohydrate treatments for hypoglycemia during the initial experiments using the controller configured with the fast lispro PK parameter settings (t_{max} = 33 min). (**E** and **F**) Cumulative BG concentrations (E) and simulated profiles (F) from the nine subjects participating in the repeat experiments using the controller configured with the slow PK settings (t_{max} = 65 min). Model-estimated insulin profiles are depicted by the black hatched curve for the fast PK parameter settings in (B) and (D) and for the slow PK parameter settings in (F).

partially treat the meal) would increase insulin concentrations in a more timely fashion than the reactive algorithm alone (*13*).

Insulin analogs with more rapid PK and less variability than lispro are desirable, as they would be expected to lower glucose excursions after meals while also reducing the risk of late postprandial hypoglycemia. In the absence of faster insulin analogs, we have shown that slower algorithm PK parameter settings could prevent hypoglycemia in subjects with slower lispro PK while resulting in only a minimal increase in the aggregate mean BG (~14 mg/dl) in subjects with faster lispro PK (Table 2). This suggests that the slower PK parameters could be widely applicable. In addition to the four-fold intersubject variability in lispro PK that we observed, there was occasionally as much as a 50% intrasubject variability in repeat experiments, suggesting that any attempt to customize or tailor the algorithm's PK parameter settings to a particular individual might be futile. However, our results show that such a customization might not be necessary, as our control algorithm appears to be robust enough to permit adoption of a universal PK parameter setting that is able to provide safe, reliable, and effective BG regulation for a broad population. By choosing a t_{max} value of 33 min in the initial studies, we were able to test the lower-bound physiologically relevant value and thereby evaluate the robustness of our control algorithm in regulating BG in subjects with substantially slower lispro PK. Our initial experiments with the fast PK parameter settings allowed us to conclude that hypoglycemia was preventable as long the subject's t_{max} was not more than twice the value used by the algorithm.

We performed these proof-of-principle studies with devices that are approved by the Food and Drug Administration with the hope that if the algorithm was effective, subsequent development of a practical artificial endocrine pancreas for outpatient use would be facilitated. Although a venous BG input signal is only practical for inpatient use, it did allow us to assess the performance of our control algorithm independently of confounding factors associated with the less accurate glucose input signal of a CGM device. Other closed-loop feasibility studies that used subcutaneous insulin infusion (*12*–*15*) relied on the CGM both as the glucose input signal and as the signal used to evaluate efficacy of the performance of the control system. Our study tested automated subcutaneous insulin and glucagon dosing with a reference-quality venous BG signal that was sampled frequently enough to serve both as the input to the controller and as the output signal with which to analyze system performance. As much as possible, each of the three components constituting a closed-loop system (glucose sensor, control algorithm, and drug infusion device) should be evaluated independently of the other two. Continuous glucose monitors are rapidly evolving technologies and, at the present time, do not provide a reliable metric with which to evaluate controller logic. Our study design was intended to provide the best evaluation possible of the limits and capabilities of automated controller logic in regulating BG with subcutaneous insulin and glucagon infusion.

To prepare for the next phase in the development of an artificial endocrine pancreas, we measured interstitial fluid glucose concentrations with three commercially available CGM devices in parallel during each closed-loop control experiment and examined the differences between the laboratory-quality plasma glucose measurements and the interstitial values obtained with these CGMs. On the basis of these results and our findings in this study, future studies will use CGM data as the sole input signal to the controller. However, in future studies, we will continue to use frequent reference-quality venous BG sampling as the primary metric to evaluate the ability of the control system to regulate BG. The design of future studies will also more closely mimic the conditions under which a practical closed-loop device would have to operate. Our subjects were studied in a controlled environment in which they were sedentary and ate standardized meals, albeit with a high carbohydrate content. The performance of the control system during free activity and aerobic exercise, which will provide a further challenge to the controller in terms of avoiding hypoglycemia, will be explored. Our current results suggest that automated closed-loop control of BG concentrations to the near-normal range without the need for frequent monitoring and injections, and without risk of hypoglycemia, will be feasible with a bihormonal artificial endocrine pancreas.

MATERIALS AND METHODS

Subjects

The research protocol was approved by the Massachusetts General Hospital and Boston University Human Research Committees, and all participants gave written informed consent. Subjects were required to be 18 years of age or older and diagnosed with type 1 diabetes at least 5 years before enrollment. They had to have a HbA1c of <8.5%, have body mass index between 20 and 31 kg/m^2, and be treated with an insulin pump with a total daily insulin dose of <1 U/kg. Potential subjects were excluded if their C-peptide after a mixed-meal challenge was >0.03 nM (*1*). Other exclusion criteria are detailed in SM Note 4.

Closed-loop BG control system

Insulin administration was governed by a customized MPC algorithm. In the standard MPC cost function, one term represents the objective to keep predictions of the glucose concentration (output) near a set point, and a second term represents a summation quantity that grows with the magnitude of successive variations in insulin doses (input). The input term, which determines how aggressively standard MPC is working to regulate glucose, is multiplied by a penalty that determines the emphasis placed on minimizing it relative to the output term. The mathematical expression of the MPC cost function is based on a relation between the glucose concentration and insulin doses, for which we use a linear empirical input-output mathematical model.

In light of the substantial time delay associated with subcutaneous insulin PK, standard MPC must be customized to account for pending insulin action from doses as they accumulate in the subcutaneous space as well as the compounded effect of insulin doses as they diffuse into the blood. Failure to account for the accumulation of insulin in the subcutaneous tissue as well as in blood will render any glucose control algorithm prone to excessive "stacking" of insulin and may lead to hypoglycemia. To address this, we customized standard MPC by augmenting the output term with a second output term representing the coupled accumulation of insulin in the subcutaneous depot (pending amount from successive doses) and in blood (diffused amount from successive doses), which are both functions of insulin PK. By introducing a relative "augmentation ratio" between the original and augmented output terms, a tuning parameter was created that can be used to vary the relative emphasis on either term in the optimization process. A high augmentation ratio increases the cost associated with stacking insulin, which will result in a tendency of the algorithm to refrain from administering more insulin until past insulin doses have decayed.

To facilitate the augmentation, we developed a two-compartment mathematical model for insulin PK to relate insulin doses with their accumulation in the subcutaneous depot and in blood. Insulin PK was modeled with a biexponential fit, in which the two time constants appearing in the arguments of the exponentials represent the time, t_{max}, required for a subcutaneous dose of insulin to peak in the blood and the time, $t_{95\%}$, for 95% of the dose to be cleared from blood. For each administered dose, the insulin accumulation in the subcutaneous tissue and in blood is determined and tracked by the algorithm over a time horizon equal to $t_{95\%}$ into the future. As such, the insulin dose computed at each time step is based on the aggregate insulin accumulation that is summed over all doses administered over a receding horizon equal to $t_{95\%}$ in the past. The PK parameters were initially set for a t_{max} of 33 min and a $t_{95\%}$ of 3.25 hours. These parameter values were felt to be reasonable for human experiments because they were found to give good results in preclinical experiments in diabetic swine (*17*, *18*) and because t_{max} was within the range reported by the manufacturer in the package insert for lispro (t_{max}, 30 to 90 min). After subjects with slower lispro PK developed hypoglycemia, a set of repeat experiments was performed for which the PK parameter settings were modified to a t_{max} of 65 min and a $t_{95\%}$ of 6.5 hours.

Subcutaneous doses of glucagon were computed using a PD algorithm that was triggered when BG dropped below set point or was within range and rapidly descending. Glucagon doses were computed in light of an online accumulation term that estimated the pending effect from recent glucagon doses. Estimations of the duration of action of subcutaneous doses of glucagon as well as the gains in the PD algorithm were based on pharmacodynamic and closed-loop control studies in diabetic swine (*17*, *18*, *21*). Accounting for subcutaneous accumulation of glucagon is less crucial than for insulin because subcutaneous glucagon has a more rapid effect on BG (presumably in large part due to faster PK), which was evident in our preclinical studies (*17*, *18*) and was reaffirmed in the glucagon PK analysis of this study. The potential consequences of delayed glucagon absorption pose less of a problem than delayed insulin absorption because glucagon doses serve to raise the BG concentration, which works in favor of safe BG control. Individual insulin and glucagon doses were limited to maximum values that were a function of subject weight and never exceeded 2 U for lispro and 20 μg for glucagon in any 5-min dosing interval.

The control algorithm required only the subject's weight for initialization and BG values every 5 min for online operation. Except for the change in PK parameters described above, the same algorithm parameters were used for all experiments. The control algorithm was implemented in MATLAB (The MathWorks) and ran on a Powerbook computer (Apple). For the mathematical formulation of the algorithm, see SM Notes 1 and 2 and (*17*, *18*).

Closed-loop BG control experiments

Subjects were admitted to the Massachusetts General Hospital Clinical Research Center at 12:30 p.m. and continued to receive basal insulin from their own pump until 3:00 p.m. when closed-loop control was begun. Intravenous catheters were inserted into each arm for blood sampling. One catheter was connected to a device (GlucoScout, International Biomedical) that measures plasma glucose concentrations by automatically sampling venous blood and assaying it with glucose oxidase chemistry. The other catheter was used to obtain blood samples for later measurement of plasma lispro and glucagon concentrations and for at least hourly BG measurements with the YSI 2300 STAT Plus Glucose Analyzer (YSI Life Sciences). Hourly paired GlucoScout and YSI values were required to be in agreement by International Organization for Standardization criteria (*22*).

Insulin lispro (Humalog, Eli Lilly) and glucagon (Eli Lilly) were delivered using infusion pumps (Deltec Cozmo, Smiths Medical), which were connected to subcutaneous infusion sets (Cleo 90, Smiths Medical) inserted into the abdomen. Because the minimum bolus size that could be delivered by the infusion pumps was 0.05 U, insulin lispro was delivered by two pumps: one containing U-100 lispro and the second containing U-10 lispro (diluted with Sterile Diluent for Humalog) to allow insulin dosing resolution of 0.005 U. Glucagon, reconstituted according to the manufacturer's instructions, was administered via a third pump with a dosing resolution of 0.5 μg. Each subject, therefore, had three infusion sets placed: one for U-100 lispro, one for U-10 lispro, and one for glucagon. Across all 20 experiments, the aggregate mean total daily dose of U-10 lispro was 11.7 ± 3.5% (6.6 to 20.4%) of the aggregate mean total daily dose of U-100 lispro. On average, nearly 10 times as much lispro was delivered at the U-100 concentration as at the U-10 concentration, although they were delivered in about the same overall fluid volume.

Venous BG values were obtained every 5 min from the GlucoScout (or YSI in the event that the GlucoScout was offline) and entered manually by a Clinical Research Center nurse into the computer by means of a graphical user interface. Insulin and glucagon doses calculated by the control algorithm were then displayed and entered manually into the pumps by a Clinical Research Center nurse and administered to the subject. The accuracy of glucose data entry was confirmed post hoc by comparing data stored by the control algorithm with the paper tape produced by the GlucoScout in real time during the experiment. At each 5-min sampling interval, the actual pump reservoir volumes were cross-checked by the nurses with the expected reservoir volumes, which were updated and displayed by the control algorithm after each dose was delivered. A schematic of the control system is shown in fig. S1.

Subjects were fed three meals with specified size and macronutrient content to total 30 kcal/kg per day for men and 25 kcal/kg per day for women. Subjects completely consumed each meal in 30 min. The percentages of calories provided as carbohydrate were 45% at dinner, 60% at breakfast, and 50% at lunch (SM Note 5). The meals were designed to provide a large carbohydrate challenge. An 80-kg male would receive 122 g of carbohydrate at dinner and 90 g of carbohydrate at breakfast and lunch. Other than the meals provided, subjects were not allowed to consume any other food items or drinks besides water or "diet" drinks that contain negligible calories. There were no snacks.

Hypoglycemia was defined as any plasma glucose concentration of <70 mg/dl. Oral carbohydrates (15 g) were given for treatment of hypoglycemia if the BG remained <60 mg/dl for a 20-min period and <50 mg/dl for a 10-min period or if subjects were symptomatic (SM Note 6).

Laboratory analyses

Blood for insulin and glucagon measurements was drawn into tubes containing EDTA and put immediately on ice. Plasma was isolated by centrifugation at 4°C and frozen within 30 min from the time of sampling. Insulin and glucagon were measured by im-

munoassay (Architect insulin assay, Abbott Laboratories and Millipore, glucagon assay, respectively). During screening, blood was obtained for HbA1c measurement by high-performance liquid chromatography (*23*).

PK analyses

Plasma insulin and glucagon concentrations were initially measured in samples drawn at 10-min intervals, but the interval was increased to 30 min for insulin and 20 min for glucagon because analyses demonstrated no substantive loss of information. Models for PK behavior of lispro and glucagon were derived in each subject by fitting a summation of the exponential accumulation and decay functions for each bolus to the measured insulin lispro and glucagon concentrations using a least-squares minimization protocol (SM Note 3). The t_{max} and $t_{95\%}$ values were derived from the fitted function for each subject. These values were calculated post hoc and were not available to the control algorithm.

Statistical analyses

The main outcomes were mean BG achieved, number of carbohydrate-treated hypoglycemic events, nadir BG during each experiment, percent of time in prespecified BG ranges, and a comparison between measured and model-estimated lispro kinetics. Although the controller came online with fixed parameters that automatically adapted after initialization with the subject's weight (*17*, *18*), study results were calculated for the last 24 hours of each 27-hour experiment to reduce the influence of preexperimental conditions on the outcome measures. The mean BG achieved over 24 hours was extrapolated to calculate the HbA1c expected if equivalent BG control was maintained over a 3-month period (*24*). A hypoglycemic event started when the BG fell to <70 mg/dl and ended when the BG returned to >70 mg/dl. Statistical analyses were performed in Excel (Microsoft). Comparisons between groups were performed using the unpaired sample, unequal variance (heteroscedastic) Student's *t* test.

SUPPLEMENTARY MATERIAL

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Methods

Fig. S1. Depiction of the bihormonal closed-loop control system used in the clinical trial.
Fig. S2. First closed-loop experiment in #108 using controller with fast PK parameter settings.
Fig. S3. First closed-loop experiment in #110 using controller with fast PK parameter settings.
Fig. S4. First closed-loop experiment in #117 using controller with fast PK parameter settings.
Fig. S5. First closed-loop experiment in #119 using controller with fast PK parameter settings.
Fig. S6. First closed-loop experiment in #126 using controller with fast PK parameter settings.
Fig. S7. First closed-loop experiment in #128 using controller with fast PK parameter settings.
Fig. S8. First closed-loop experiment in #115 using controller with fast PK parameter settings.
Fig. S9. First closed-loop experiment in #121 using controller with fast PK parameter settings.
Fig. S10. First closed-loop experiment in #122 using controller with fast PK parameter settings.
Fig. S11. First closed-loop experiment in #129 using controller with fast PK parameter settings.
Fig. S12. First closed-loop experiment in #132 using controller with fast PK parameter settings.
Fig. S13. Second closed-loop experiment in #115 using controller with slow PK parameter settings.
Fig. S14. Second closed-loop experiment in #121 using controller with slow PK parameter settings.
Fig. S15. Second closed-loop experiment in #122 using controller with slow PK parameter settings.
Fig. S16. Second closed-loop experiment in #129 using controller with slow PK parameter settings.
Fig. S17. Second closed-loop experiment in #132 using controller with slow PK parameter settings.
Fig. S18. Second closed-loop experiment in #110 using controller with slow PK parameter settings.
Fig. S19. Second closed-loop experiment in #117 using controller with slow PK parameter settings.
Fig. S20. Second closed-loop experiment in #126 using controller with slow PK parameter settings.
Fig. S21. Second closed-loop experiment in #128 using controller with slow PK parameter settings.
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