Supplementary Data

Russell *et al.* – Blood glucose control in type 1 diabetes with a bihormonal bionic endocrine pancreas.

1 Regulatory Oversight

In addition to IRB oversight from both the Partners Human Research Committee (Massachusetts General Hospital Institutional Review Board) and the Boston University Institutional Review Board, this study was conducted under the United States Food and Drug Administration (FDA) Investigational Device Exemption (IDE) application #G100062, approved by the Center for Devices and Radiological Health. We received an Investigational New Drug (IND) Exemption from the FDA for the use for glucagon in a pump for up to 27 hours.

2 Subjects

Major additional exclusion criteria included pregnancy or sexual activity without use of contraception, history of impaired gastric motility requiring treatment, anemia, renal insufficiency, abnormal thyroid function, elevated alanine amino transferase, untreated or inadequately treated hypertension, coronary artery disease, heart failure, or seizures, use of medications for PG control other than insulin or medications that affected gastric motility, history of aspirin allergy, aspirin intolerance, or peptic ulcer, and inability to perform at least 30 minutes of moderate exercise.

3 Closed-loop Glucose Control Algorithms for Insulin and Glucagon Dosing

With the exception of a weight-based partial meal priming bolus, delivered at the beginning of each meal, insulin and glucagon were administered by a fully automated system (Supplementary Fig. 1) using control algorithms very similar to those previously described.[1] The only input signal to the control algorithm at every five-minute interval was the most recent Navigator CGM glucose value in that five-minute sampling window. Insulin dosing was controlled by a customized model predictive control (MPC) algorithm incorporating a pharmacokinetic (PK) model for insulin lispro that assumed a t_{max} of 65 minutes and a clearance time of ~ 6.5 hours, which are the same values that were used in the repeat experiments of our previous study. In computing the insulin dose, the MPC algorithm uses a five-minute glucose predictor along with a prediction of the pending effect of every insulin dose as far out as ~ 6.5 hours in the future. Glucagon dosing was controlled by a customized proportional-derivative (PD) algorithm.[1] However, two main modifications were made to these control algorithms. These are described below.

In the insulin algorithm, the basal rate of insulin, which is automatically set and determined by the algorithm, was allowed to modulate continually between 0 U/hr and an upper bound that is a function of subject weight. The basal rate modulation was automatically performed by the algorithm online in response to the glucose level and its rate of change. This added flexibility allowed the insulin algorithm to temporarily suspend insulin administration (i.e. modulate the basal rate to 0 U/hr) even at a hyperglycemic level whenever there was a rapid decline in the glucose level. Similarly, it also provided a temporary shut off in insulin administration whenever the glucose level was near or below the low end of normal range. This is essentially analogous to the so-called "low glucose suspend" capability, except that the algorithm may automatically invoke it and reverse it by resuming insulin administration as frequently as the glucose level dictates online. Overall, this modification adds versatility to the algorithm to provide safer and smoother glucose control.

In the glucagon algorithm, the proportional and derivative gains were reformulated to be functions of the glucose level, allowing them to dynamically change online in response to the glucose level. This modification allows the algorithm to raise its glucagon gains and issue larger glucagon doses whenever the glucose level was near or below the low end of normal range, and conversely reduce its glucagon gains and issue smaller glucagon doses whenever the glucose level was near the high end of normal range. Furthermore, the overall computed glucagon was occasionally increased further by an outer scaling parameter whenever the algorithm-estimated plasma insulin levels exceeded a threshold ratio relative to the algorithm-estimated baseline plasma insulin levels. The added outer scaling parameter acts occasionally to further increase glucagon dosing to counter impending hypoglycemia or reverse an episode of hypoglycemia, and offers distinguished glucagon dosing responses among situations of similar low and/or declining glucose levels, depending on the estimated plasma insulin on board. These refinements to the glucagon algorithm were motivated by our analysis of hypoglycemic episodes that occurred during our first human study.[2]

4 Automated Glucose Control Experiments

Subjects were asked to eat breakfast at home and finish the meal by 8:00 AM, bolusing insulin for the meal using their insulin pump as usual, and arriving for admission by 10:00 AM of the first day.

All subjects were given an 81 mg baby aspirin to be chewed at the beginning of the study and then daily in order to help prevent occlusion of IV lines. The GlucoScout device was primed and calibrated according to the manufacturer's instructions except that no heparin was added to the flush bag.

The Navigator session was started and the initial calibration was requested by the device between 11:00 AM and 12:00 PM. All Navigator calibrations were performed using plasma glucose (PG) values reported by the GlucoScout. This was accomplished using the Navigator "cradle", an investigation device that allows the Navigator to accept calibrations through a computer interface rather than solely through the built-in Freestyle meter. The Navigator cradle also allows the CGM glucose (CGMG) to be streamed directly to a computer every five minutes.

A lunch meal was provided between 11:30 AM and 12:00 PM and was treated with insulin by the subjects as usual. A second Navigator calibration was requested approximately two hours after the first, between 1:00 and 2:00 PM. Subjects continued their normal basal insulin infusion through their own pump until 3:00 PM when their pump was removed and the closed-loop experiment began.

An Insulet Omnipod was filled with glucagon (Lilly) reconstituted according the manufacturer's instructions. In accordance with our IND Exemption, allowing the use of glucagon in a pump for up to 27 hours, the glucagon pod was replaced once during the experiment, at 3:00 PM on the second day. Commercially available glucagon is more vulnerable than insulin to deamidation, hydrolysis to shorter peptides, and formation of amyloid fibrils.[3–5] Its stability in solution, therefore, is not sufficient for multi-day use in a pump reservoir. However, it has previously been shown in human experiments that the anti-hypoglycemic effect of this glucagon formulation given at microdoses is retained for up to 27 hours after reconstitution in a pump reservoir without any apparent loss of efficacy.[1,2,6] In experiments performed in porcine models of diabetes, we have shown that the anti-hypoglycemic effects of this glucagon formulation are retained for up to seven days after reconstitution.[7] The retention of biological activity in the face of instability suggests that shorter peptides produced by hydrolysis of full-length glucagon may have equal or possibly greater anti-hypoglycemic activity than the intact peptide.[3] Regardless, because the commercially available formulations would not pass FDA stability requirements for use in a pump, a stable form of glucagon is needed. At least three such formulations have been reported.[3–5,8] An Insulet OmniPod was filled with insulin lispro. A single insulin pod was used throughout each experiment except in cases of an insulin delivery failure (noted in Supplementary Figures 2S, 7S, and 9S). The glucagon and insulin pods were adhered to the skin of the abdomen, one on either side of the umbilicus, primed, and activated according the manufacturer's instructions. Insulin delivery failures occurred on three occasions. It is not clear what the source of these failures was, but all were later confirmed by offline analysis of plasma insulin levels (Supplementary Figures 2S, 7S, and 9S). All doses

issued by the algorithm were confirmed to have been received by the pods in all three instances. In at least one instance, the skin under the insulin pod was damp and there was the characteristic odor of the preservative used in insulin preparations, suggesting that the catheter may have pulled out of the skin and come to rest between the pod and the skin.

The final Navigator calibration was requested by the device between 1:00 AM and 2:00 AM of the second day. In one experiment this calibration schedule was delayed (Supplementary Figure 4S) such that the first two Navigator calibrations occurred at 2:00 PM and 4:00 PM on the first day and the final calibration occurred at 4:00 AM on the second day. There was a provision in the protocol to force a calibration of the Navigator at 6:00 daily if the CGMG was not within the International Organization for Standardization (ISO) standard compared with PG; namely within 20% of PG if the PG $>$ 75 mg/dl or within 15 mg/dl of PG if the PG $<$ 75 mg/dl. However, this calibration was never required according to these criteria and no calibrations other than the standard calibrations requested by the Navigator were done. Therefore, the final 41 to 42 hours of the experiments were performed without any Navigator calibrations. The Navigator had a 100% data reporting efficiency in 9 of the 12 experiments (glucose values were reported every 5 minutes for 51 continuous hours). In each of the remaining 3 experiments, the Navigator reported at all but a single 5-minute interval (corresponding to a 99.9% data reporting efficiency). In those three isolated instances, the algorithm issued a basal dose of insulin that was calculated by the control algorithm according to (1) the current online basal rate (which was determined automatically by the algorithm) and (2) the rate of fall in glucose entering the dropout interval.

The subjects consumed dinner at 6:00 PM on the first and second day, breakfast at 7:30 AM on the second and third days, and lunch at 12:30 PM on the second and third days for a total of six meals. On the second day, each subject participated in a period of structured exercise that began at 4:00 PM and ended at approximately 4:30 PM.

During closed-loop control a CGMG measurement was streamed via the Navigator cradle to a laptop computer residing on a modified IV pole. The control algorithm commanded doses of insulin and/or glucagon via two separate OmniPod Personal Diabetes Manager devices that were hardwired to the laptop. These communicated to the insulin and glucagon pods on the subject via radio frequency transmission. After each dose, the pods communicated their remaining reservoir volumes back to the control system for dose reconciliation. The actual dose delivered was then plotted and taken into account in future dosing. If a dose failed at one time step, as occasionally happened, the dose delivered in the next time step was calculated with this knowledge. A high-capacity battery backup power supply was mounted on the IV pole so that the apparatus did not need to be tethered by a power cord. The subjects were free to ambulate around the floor as long as they remained within 2–3 feet of the IV pole.

The GlucoScout device was also mounted on the IV pole and sampled blood automatically for PG every 15 minutes. The device uses a closed sterile fluid circuit and the sampled blood was re-infused after every measurement, so there was no net blood loss associated with PG sampling. Blood samples for YSI verification of PG measurements, and for later analysis of plasma insulin and glucagon levels, were drawn from a side port on the sampling line during the measurement phase of the GlucoScout cycle (which lasted $\sim$ 50 seconds) so that only a single IV was required.

The menu for meals was agreed upon by the subjects and the research nutrition service prior to their first experiment. The meals were required to provide at least 24 kcal/kg/day for males and 20 kcal/kg/day for females, with subjects encouraged to plan meals providing 30 kcal/kg/day for males and 25 kcal/kg/day for females. A minimum carbohydrate intake per meal was based on a macronutrient content of 50% carbohydrate with no less than 33% of the total daily carbohydrates consumed in any one of the three daily meals. The maximum carbohydrate content for a single meal was 50% of the total estimated daily carbohydrate requirement, thereby allowing subjects to eat up to 150% of the minimum daily required carbohydrate intake. Subjects ate meals with the same macronutrient content at each meal for both study visits. The subjects were required to finish their meals in a period of 30 minutes. Meals were weighed at the time of serving and after the subject was finished and the total calories and macronutrient content was documented for each meal.

The subject participated in a period of exercise starting at 4:00 PM on the second day. They pedaled a stationary bicycle with the goal of maintaining their heart rate between 120 and 140 beats per minute until the total number of heart beats (calculated by interpolating from heart rate documented every two minutes) was equal to 4000. This took between between 30 and 35 minutes. All subjects were able to complete the exercise according to protocol.

Exercise was scheduled at 4:00 PM on the second day in order to allow sufficient time after exercise (approximately 90 minutes) for the effectiveness of glucagon to be adequately assessed before the start of dinner. Scheduling exercise on the second day also allowed for the evaluation of the effect, if any, exercise might have on glycemia the following night. Furthermore, by scheduling exercise to begin near the midway point in the experiment, the subject will have completed 25 hours of closed-loop control and will have 26 hours of closed-loop control remaining. Thus the conditions during the 24-hour period prior to exercise were not significantly different from the 24-hour period following it.

The experiment was ended at 6:00 PM on the third day after 51 hours of closed-loop control.

5 Statistical Analysis

The main outcome measures reported in the Results section and in the first row of Table 1 are calculated from all of the pooled data from all experiments, whereas the per experiment means are calculated from all of the pooled data for that experiment. The bottom row of Table 1 shows the mean and standard deviation (SD) of each experiment ($n = 12$). For such metrics as mean PG, and PG during the nighttime hours, the means are the same for both methods of calculation because each experiment has equal numbers of points that contributed to the overall mean. However, the SD is less for the per-experiment calculations because the experimental means fall within a narrower range than all of the PG values throughout an experiment. For other outcome measures, such as time in range, carbohydrate consumption, and drug dosage, both the means and SDs could be different for the two different calculation methods because there may be different numbers of points from each subject.

The mean intrasubject difference in insulin lispro t_{max} was calculated as the mean of the differences between the t_{max} values for each subject's two experiments ($n = 6$). The mean intersubject difference was calculated as the mean of the differences between the t_{max} in each experiment and in all other experiments performed in the other subjects ($n = 120$).

The mean intrasubject difference in total daily dose (TDD) was calculated as the mean of the differences between each subject's TDD ($n = 6$). The mean intersubject difference was calculated as the means of the differences between the TDD of each subject's experiment with the smaller priming bolus and the TDD of all other subjects' experiments with the larger priming bolus, and vice versa ($n = 60$).

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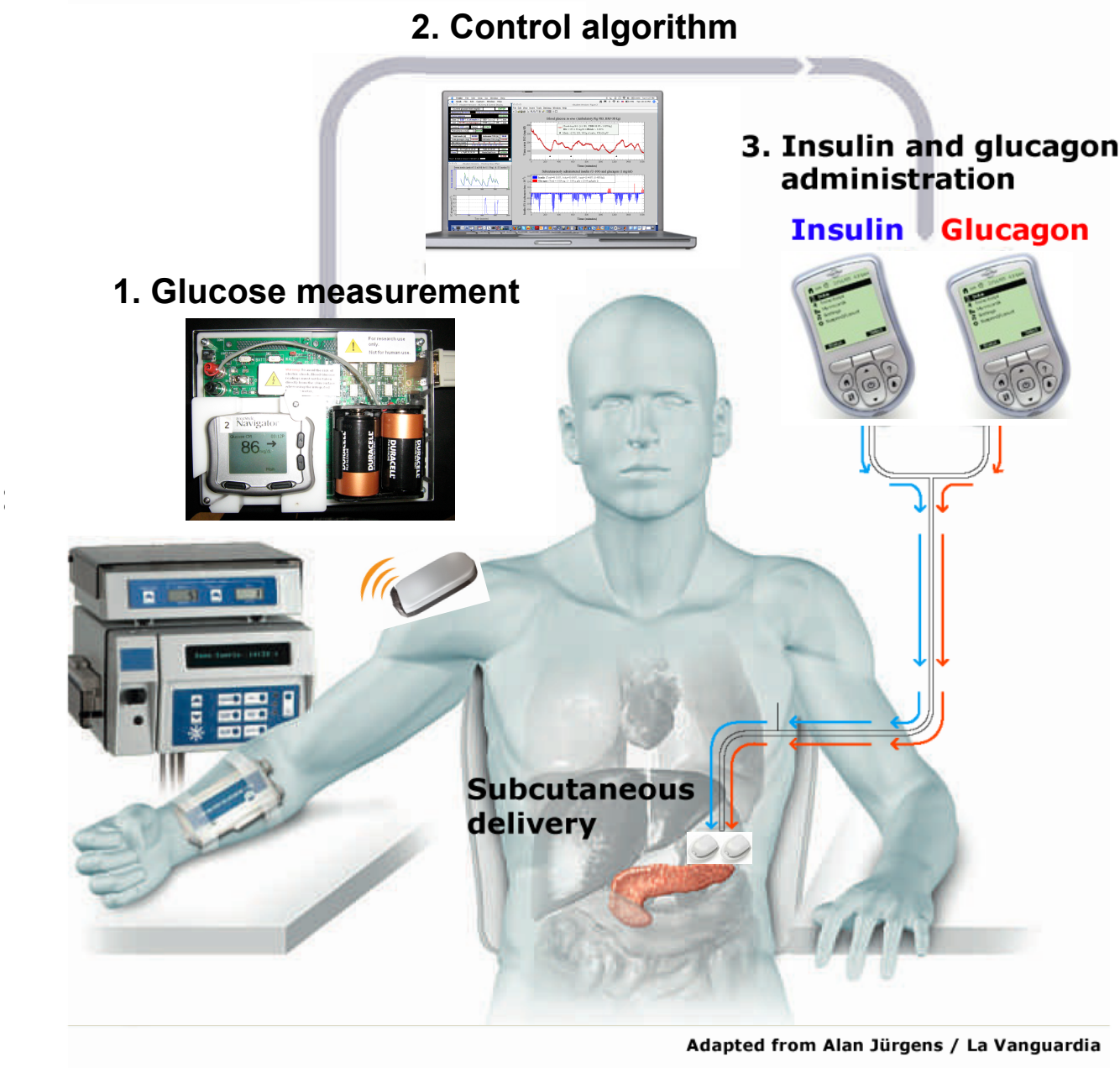

Figure 1S. Schematic of the bihormonal bionic endocrine pancreas used in the clinical trial. The control algorithm, which ran on a laptop computer (Apple), shown at station 2 (labeled Control algorithm), responded to glucose readings streamed online every five minutes from a Navigator CGM docked in the Navigator cradle (Abbott Diabetes Care), shown at station 1 (labeled Glucose measurement). Basing its calculations solely on the Navigator CGM glucose and previously administered insulin and glucagon doses, the control algorithm commanded insulin and glucagon control doses wirelessly using the OmniPod infusion system (Insulet), shown at station 3 (labeled Insulin and glucagon administration). Venous PG was also measured every 15 minutes using an FDA-approved GlucoScout (International Biomedical), shown in the lower left. Venous PG data was not provided to the control algorithm.

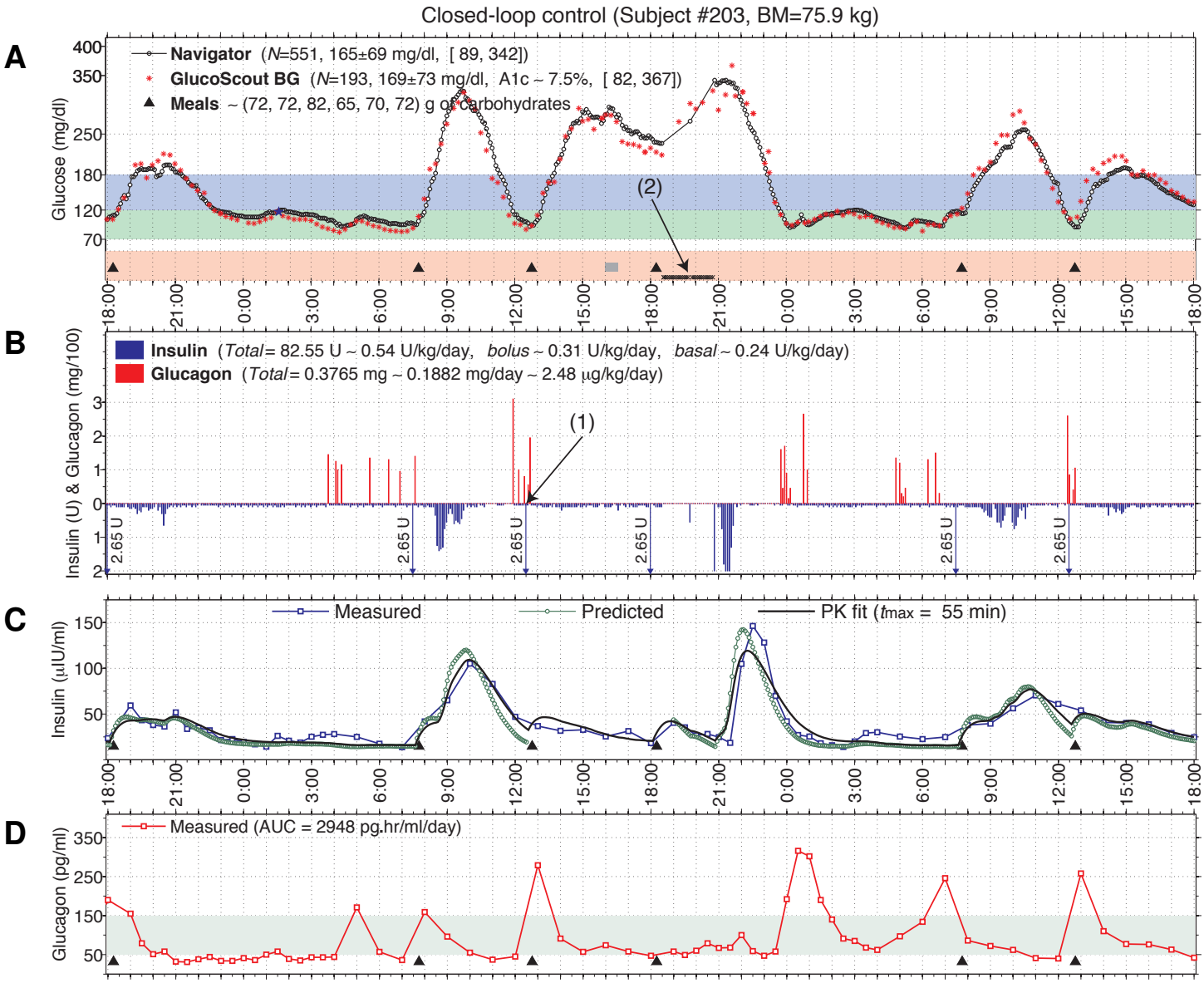

Figure 2S. 48-hour closed-loop experiment in #203 using a meal priming bolus of 0.035 U/kg. Same interpretation as Figure 1. In this experiment there was an erroneous report from the pod that 61.35 units of insulin lispro had been delivered at 12:25 on the second day, right before the meal priming bolus of 2.65 U was commanded for lunch at 12:30 (arrow labeled (1) in Panel B). This erroneous information was then incorporated into future insulin bolus dosing calculations by the algorithm, resulting in administration of only basal insulin over the next six hours despite CGMG and PG values that rose to over 250 mg/dl (note insulin dosing in Panel B from 12:00–18:00 on the second day). The system was subsequently restarted between 18:30–20:45 on the second day as dinner was ending (black $\times$'s on timeline indicated by arrow labeled (2) in Panel A shows interval when system was offline from 18:30–20:45 on the second day). During the two-hour delay while the system was being restarted, PG had risen to $>$ 350 mg/dl. Regardless, the system had the PG back to the normal range (70–120 mg/dl) approximately 2.5 hours after being restarted. The pod-reporting error was only appreciated in post-experiment analysis of the computer record of the experiment. Interestingly, the report of insulin delivered from the pod to the control algorithm went back to the correct value in the time step after the error occurred, but the large phantom dose had already been incorporated into the insulin-on-board calculations. No similar incidents have ever been observed nor could the erroneous dose report be reproduced in simulated runs using the data from this experiment. All of the data from this experiment, as in each of the cases of technical failure, were included in the calculation of outcome measures.

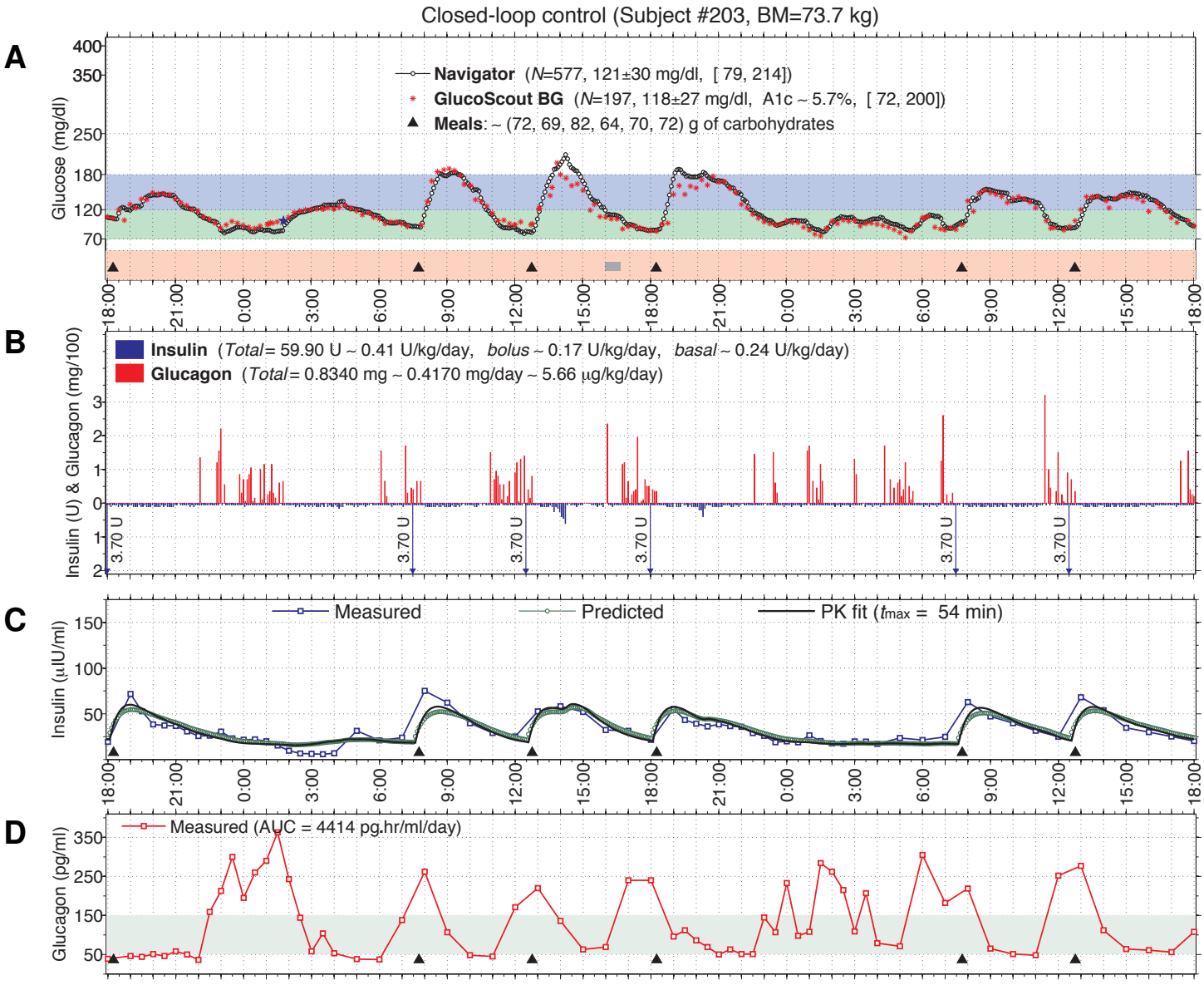

Figure 3S. 48-hour closed-loop experiment in #203 using a meal priming bolus of 0.05 U/kg.

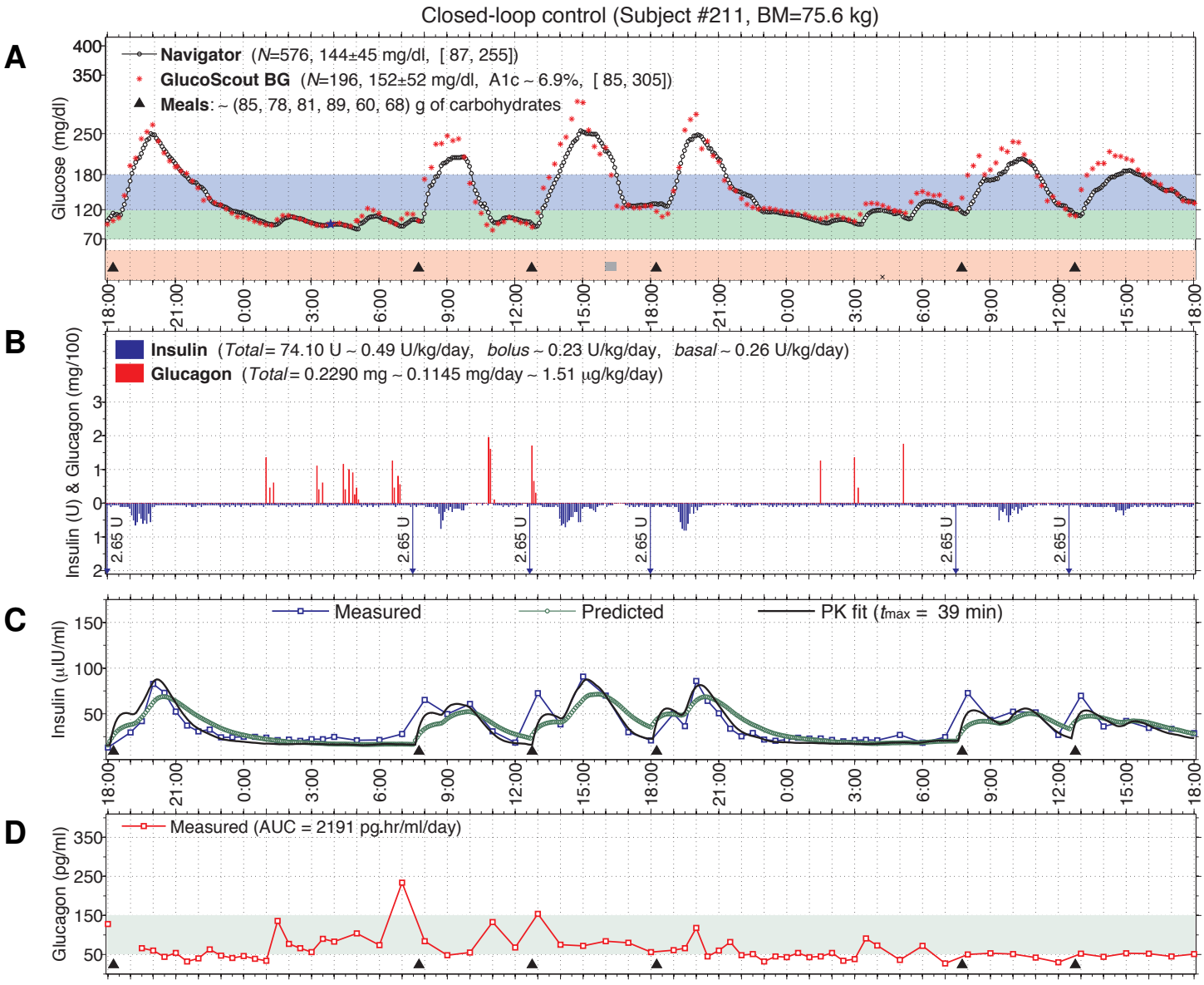

Figure 4S. 48-hour closed-loop experiment in #211 using a meal priming bolus of 0.035 U/kg.

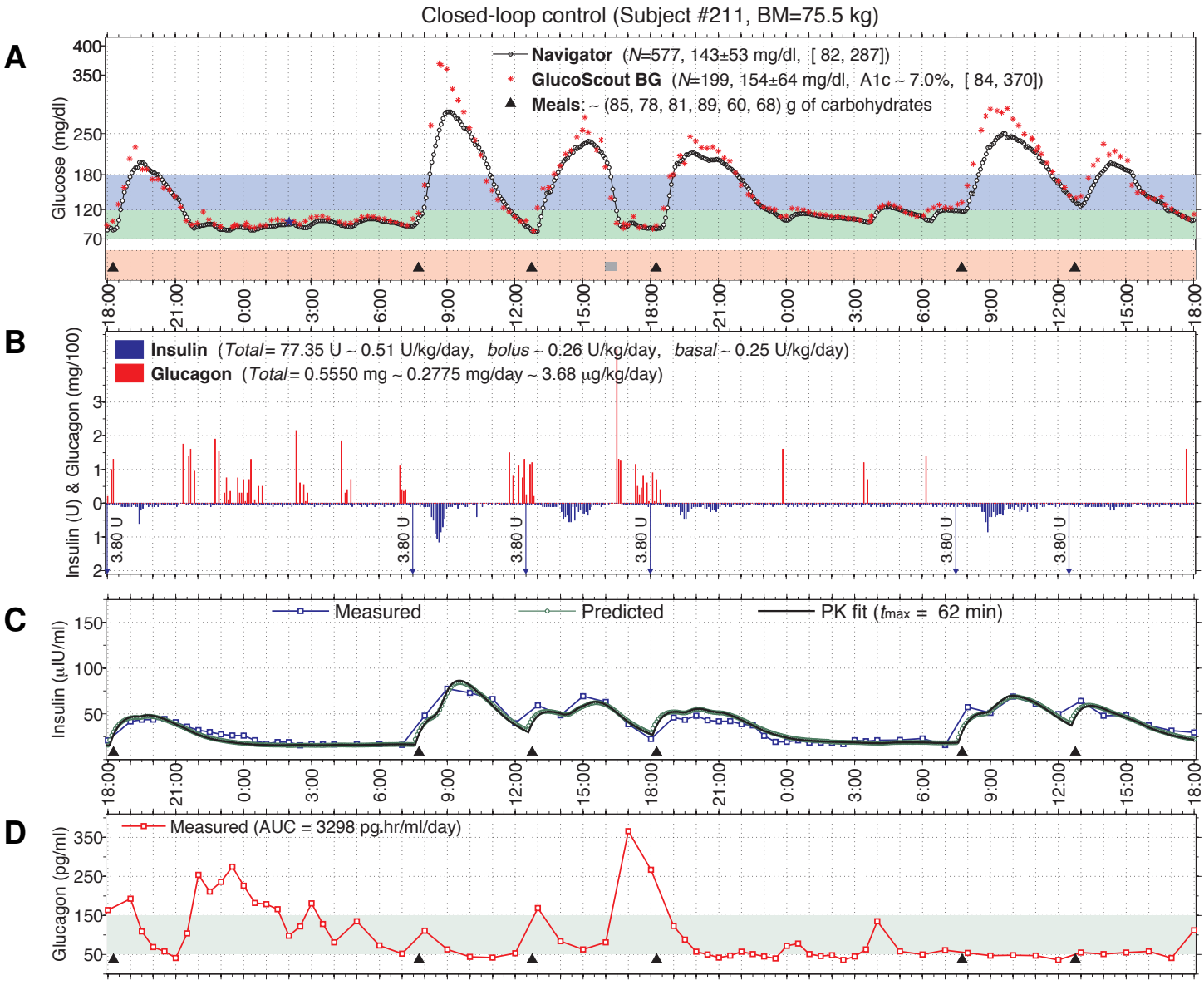

Figure 5S. 48-hour closed-loop experiment in #211 using a meal priming bolus of 0.05 U/kg.

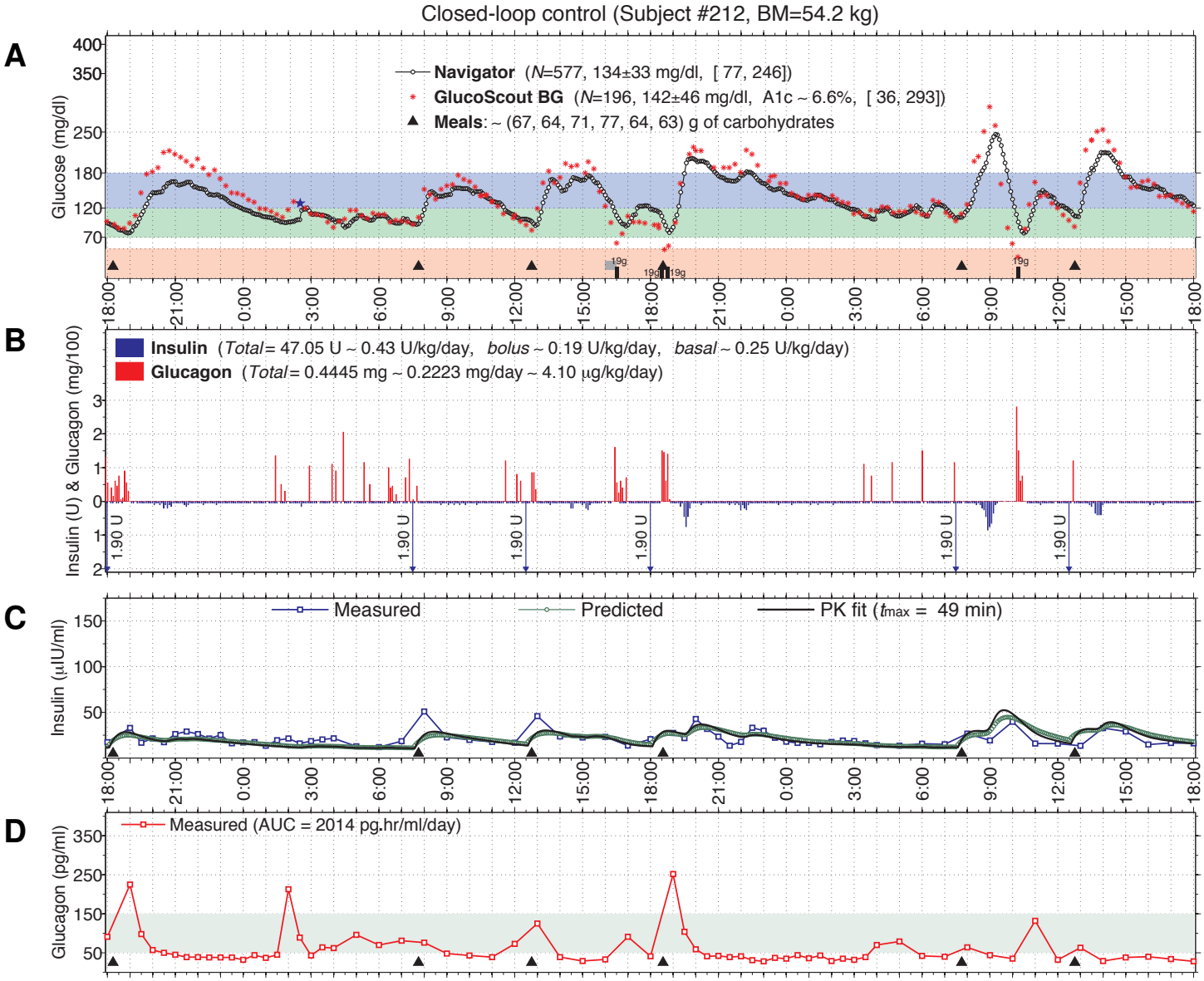

Figure 6S. 48-hour closed-loop experiment in #212 using a meal priming bolus of 0.035 U/kg. One episode of hypoglycemia in this experiment, treated with two juice interventions at 18:30 and 18:45 (indicated by small black rectangles along the timeline of Panel A and annotated with the carbohydrate content of each intervention), occurred when the meal priming bolus was delivered on schedule at 18:00 but the meal was not presented to the subject until 18:20. There were two other episodes of hypoglycemia in this experiment. Note that during all of the episodes of hypoglycemia documented by PG, the CGMG never fell below 77 mg/dl. All of the data from this experiment, despite late provision of a meal that may have resulted in one of the hypoglycemic episodes, were included in the calculation of outcome measures.

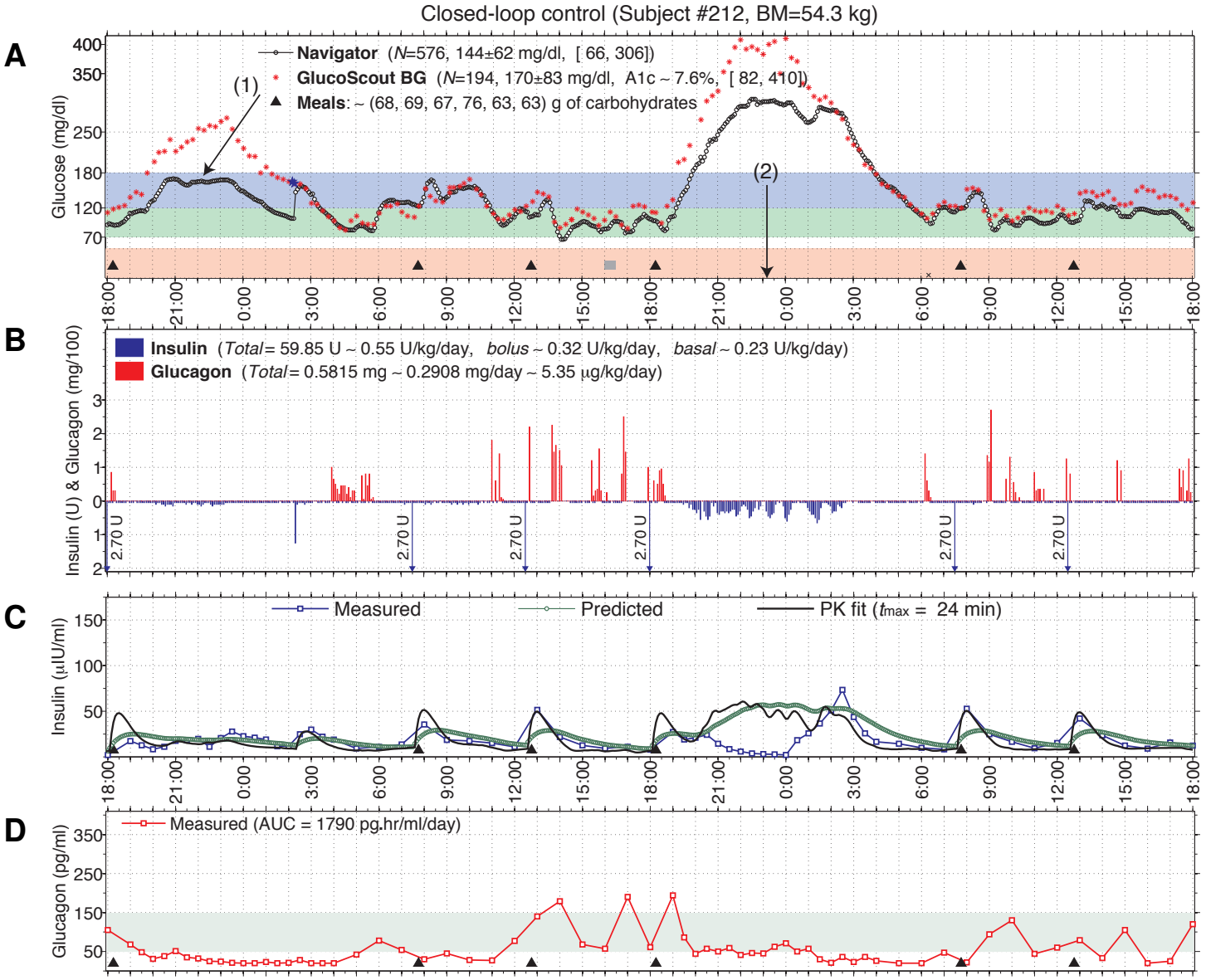

Figure 7S. 48-hour closed-loop experiment in #212 using a meal priming bolus of 0.05 U/kg. In this experiment the CGMG was underestimating PG substantially for the first eight hours of the experiment (arrow labeled (1) in Panel A). The control algorithm only receives input from the CGMG, so there was very little insulin dosing beyond basal, despite reference PG values that rose to $>$ 250 mg/dl. The disparity between CGMG and PG was corrected by the calibration routinely requested by the Navigator at approximately 2:00 on the first day. The experimental procedure was explicitly designed to avoid correction of erroneous CGMG signals except at regularly scheduled calibration times or at the daily "sanity check" performed daily at 6:00, because in real-world use, the patient would not have the benefit of frequently measured PG values to know that the CGM was in error. By performing the experiment in this manner, we provide a more realistic picture of expected system performance in actual use. This experiment was also affected by an insulin delivery failure sometime between 18:00 and 21:00 on the second day, which was clinically suspected on the basis of persistent hyperglycemia as measured by both the CGMG and PG despite what appeared to be adequate insulin dosing. The insulin pod was replaced at 23:08 (arrow labeled (2) in Panel A). The suspected insulin delivery failure was subsequently verified based on measured plasma insulin levels, which began to fall starting at $\sim$ 20:30 on the second day, despite continued dosing by the algorithm, and subsequently fell to near the limit of detection at $\sim$ 0:00 on the third day. The missed insulin delivery is evident in Panel C by the disparity between predicted insulin levels (green circles) and measured insulin levels (blue circles) between 20:30 on the second day and 1:00 on the third day. After pod replacement, the insulin levels began to rise again at $\sim$ 0:00 on the third day and the PG was brought back to the normal range (70–120 mg/dl) by 5:45 on the third day. All of the data from this experiment, as in each of the cases of technical failure, were included in the calculation of outcome measures.

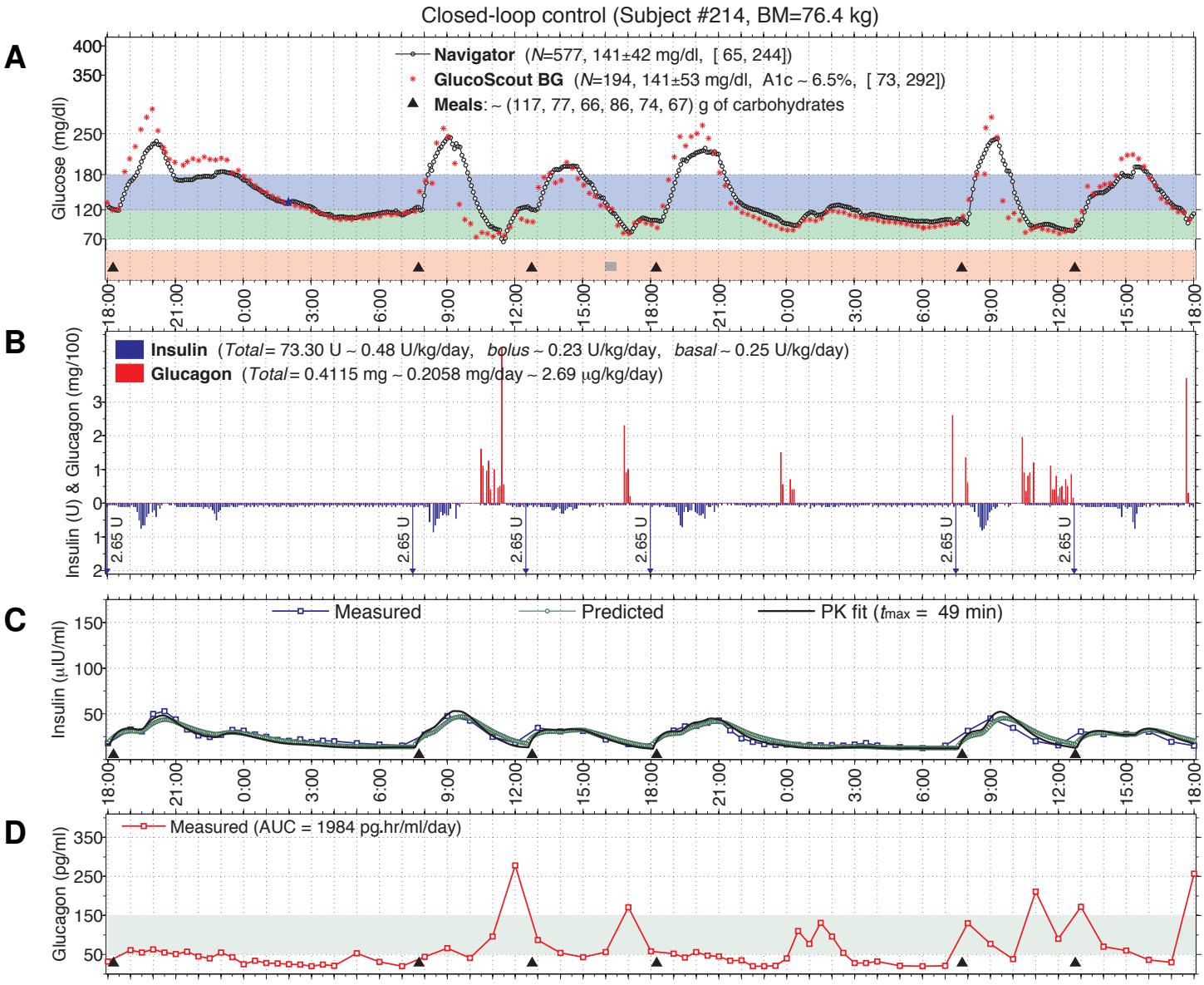

Figure 8S. 48-hour closed-loop experiment in #214 using a meal priming bolus of 0.035 U/kg.

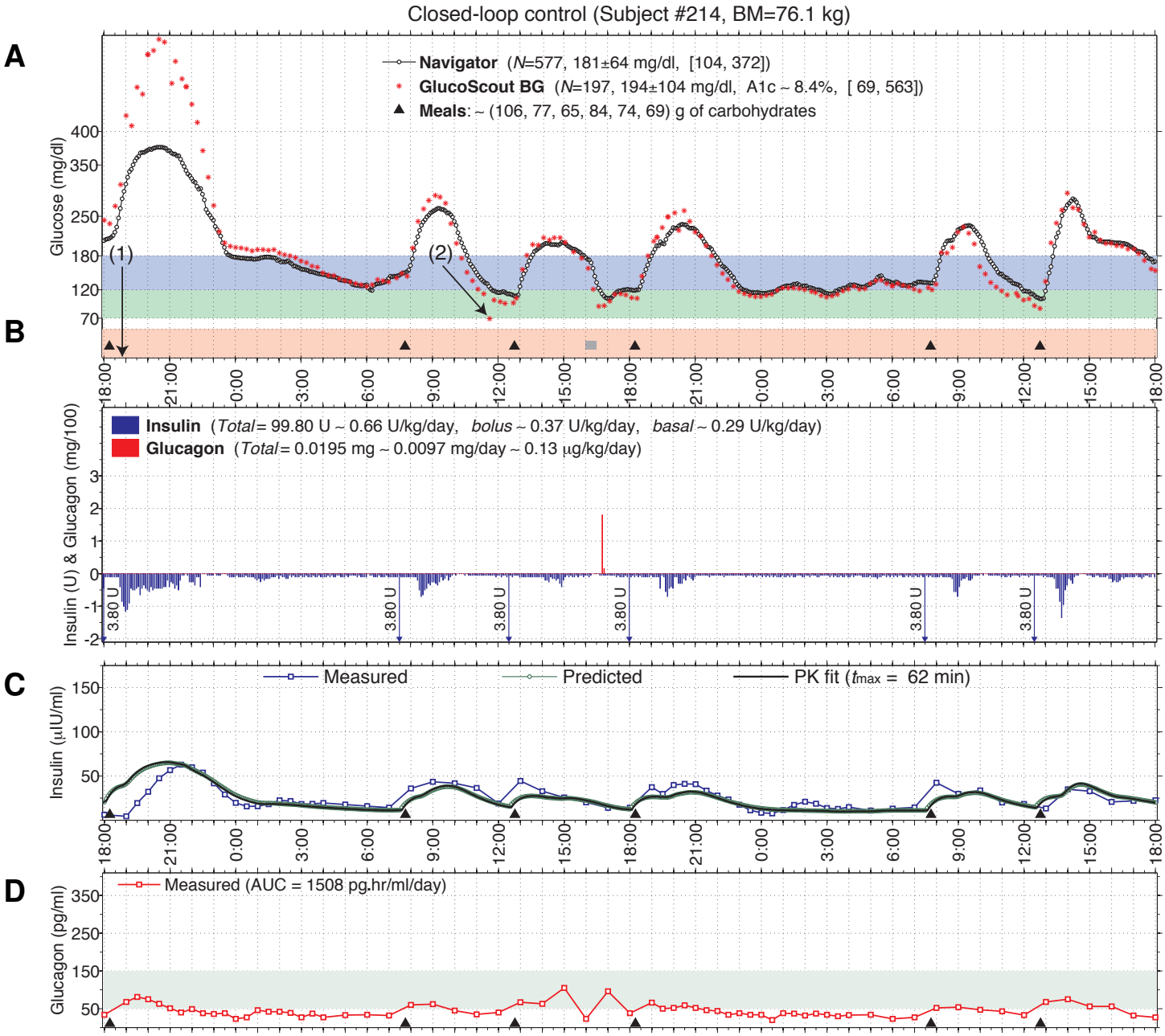

Figure 9S. 48-hour closed-loop experiment in #214 using a meal priming bolus of 0.05 U/kg. This experiment was affected by an insulin delivery failure sometime before 18:00 on the first day. This was clinically suspected on the basis of an unusual degree of hyperglycemia as measured by both the CGMG and PG, which suggested the meal priming bolus had not been delivered. The insulin pod was replaced at 18:51 (arrow labeled (1) in Panel A). The suspected insulin delivery failure was subsequently verified based on measured plasma insulin levels, which did not rise as expected in response to the meal priming bolus at 18:00 on the first day. The missed insulin delivery is shown by the disparity between predicted insulin levels (green circles) and measured insulin levels (blue circles) from 18:00 to 21:00 on the first day. After pod replacement, the insulin levels began to rise in response to dosing and the PG began to fall after 20:30 on the first day. An episode of hypoglycemia occurred in this experiment at 11:30 on the second day (arrow labeled (2) in Panel A) when the PG was measured at 69 mg/dl. It seems likely that this was PG measurement error as the PG values before and after this measurement were much higher. All of the data from this experiment, as in each of the cases of technical failure or apparent measurement error, were included in the calculation of outcome measures.

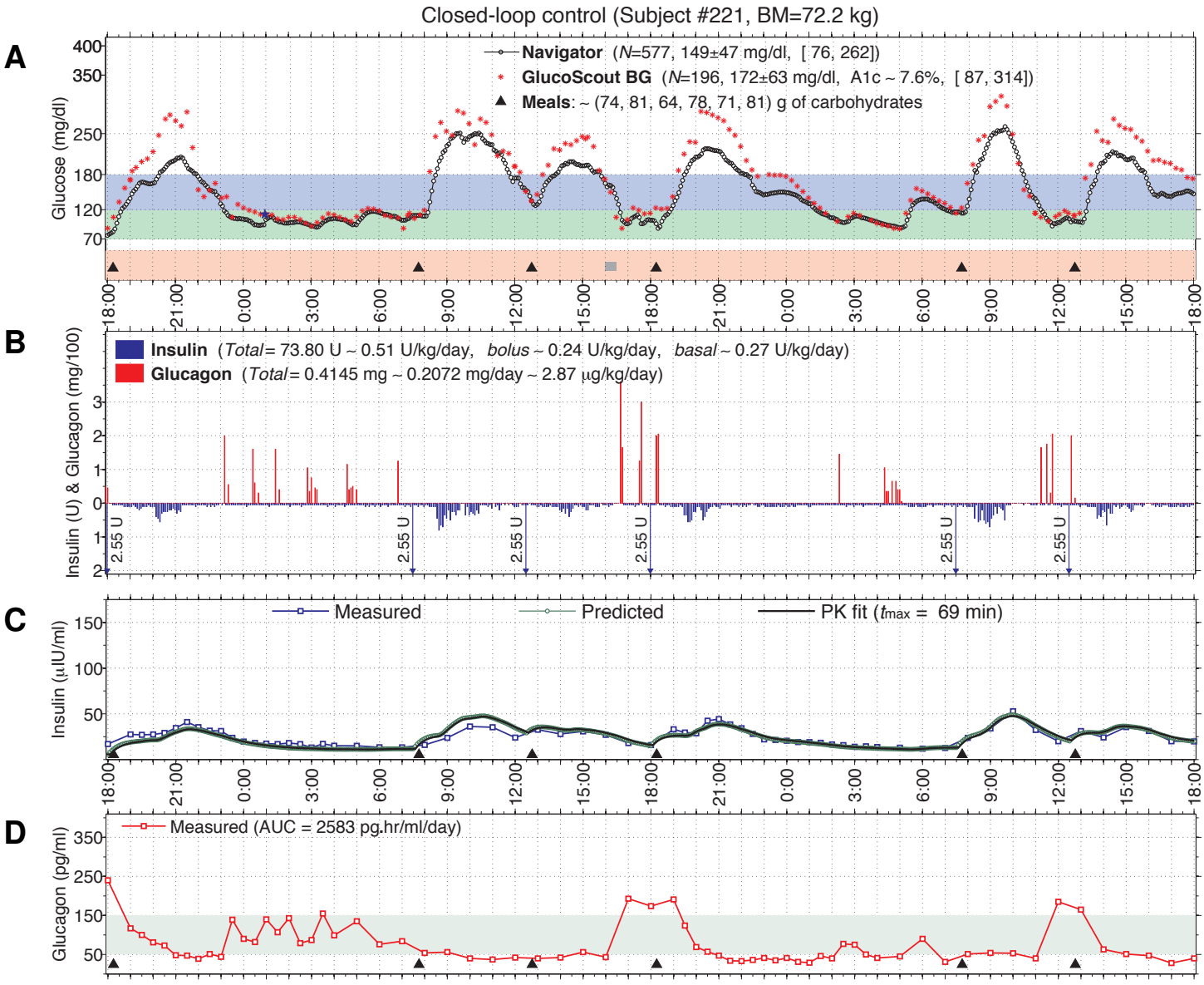

Figure 10S. 48-hour closed-loop experiment in #221 using a meal priming bolus of 0.035 U/kg.

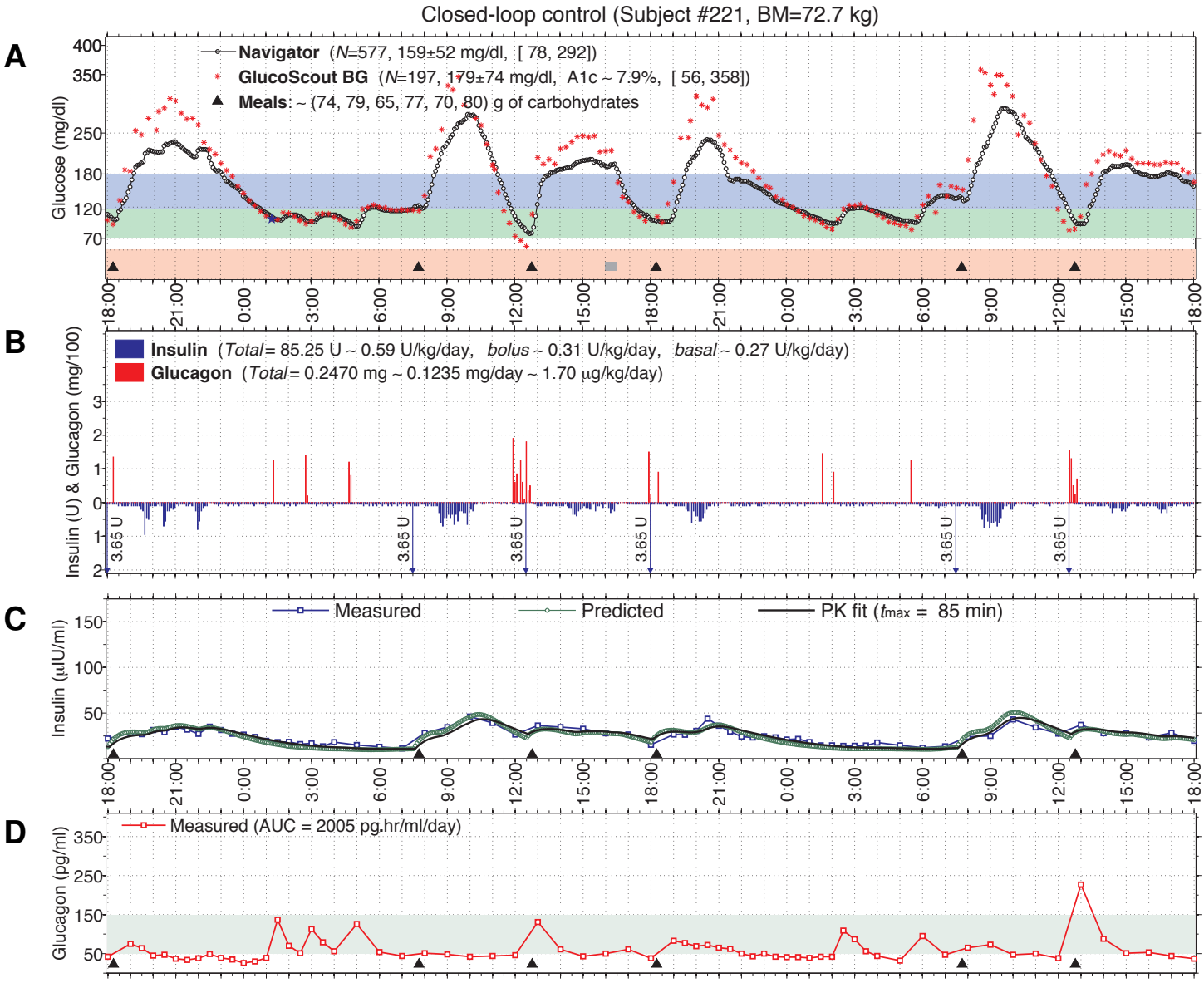

Figure 11S. 48-hour closed-loop experiment in #221 using a meal priming bolus of 0.05 U/kg. There was one episode of hypoglycemia in this experiment starting at 12:15 on the second day. The episode did not meet criteria for automatic carbohydrate intervention and the subject denied having symptoms, so the episode resolved without a carbohydrate intervention, although a meal was given 15 minutes later according to schedule at 12:30. Note that despite the episode of hypoglycemia documented by PG, the CGMG never fell below 78 mg/dl.

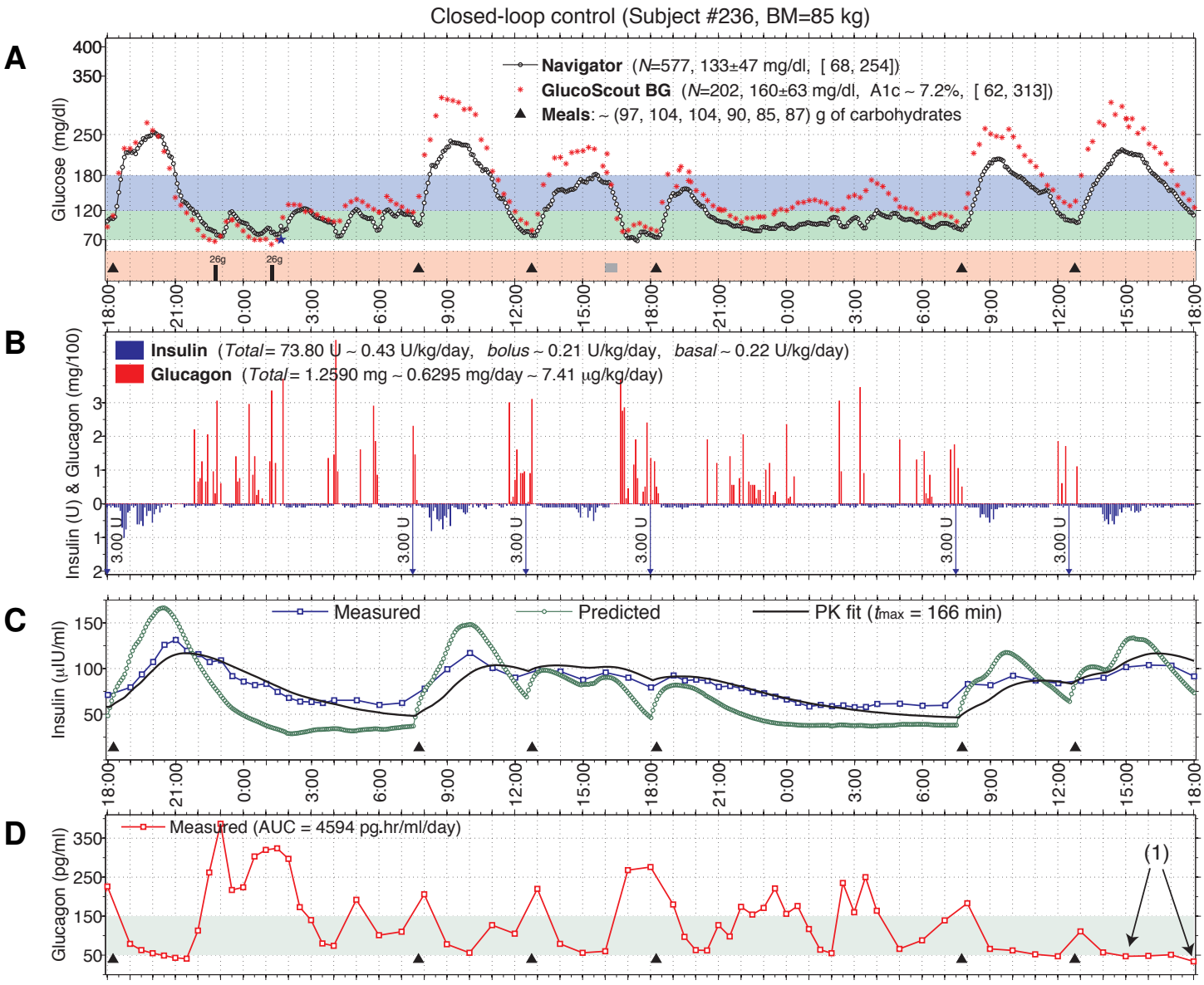

Figure 12S. 48-hour closed-loop experiment in #236 using a meal priming bolus of 0.035 U/kg. The subject complained of constipation and/or nausea from 14:30–15:30 and again at 18:00 on the third day (arrows labeled (1) in Panel D). The symptoms of nausea do not appear to be correlated with glucagon levels as the glucagon levels during this interval were the lowest of the experiment. There were two episodes of hypoglycemia that occurred at 22:45 on the first day and 1:15 on the second day (indicated by small black rectangles along the timeline of Panel A and annotated with the carbohydrate content of each intervention). These were treated with carbohydrate interventions due to symptoms of hypoglycemia, although they did not meet criteria for automatic treatment. Note that despite the episode of hypoglycemia documented by PG, the CGMG never fell below 70 mg/dl during these episodes. The CGMG fell to a nadir of 68 mg/dl in this experiment at a time when the PG was actually near 100 mg/dl. Note that t_{max} for insulin lispro for this subject was markedly higher than the mean (166 minutes in this experiment).

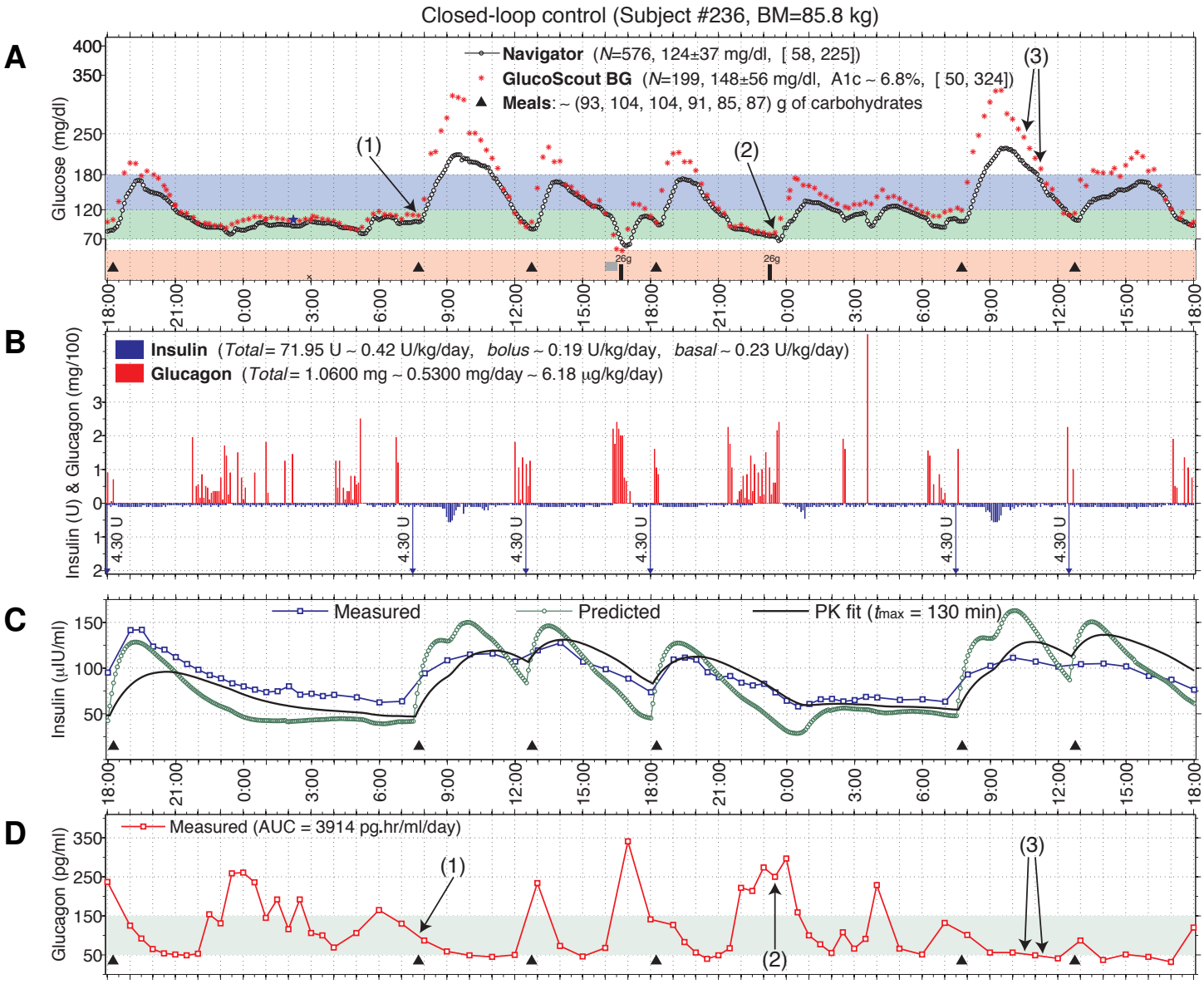

Figure 13S. 48-hour closed-loop experiment in #236 using a meal priming bolus of 0.05 U/kg. The subject complained of constipation at 7:45 on the second day (arrows labeled (1) in Panels A and D), of feeling "low and lousy" at 23:30 on the second day (arrows labeled (2) in Panels A and D), and on the third day of feeling "lousy" and "tired" at 10:30 and "lousy" at 11:15 (arrows labeled (3) in Panels A and D). The symptoms do not appear to be clearly correlated with PG or glucagon levels. There was one episode of hypoglycemia that occurred immediately following exercise, which started at 16:30 on the second day, and for which the subject received a carbohydrate intervention. This is the only episode of hypoglycemia in this study during which both the CGMG and PG simultaneously fell below 70 mg/dl. The subject also requested a carbohydrate intervention at 23:15 on the second day for symptoms of hypoglycemia, although the PG at this time was $\sim$ 80 mg/dl and stable. (The carbohydrate interventions are indicated by the small black rectangles along the timeline of Panel A and are annotated with the carbohydrate content of each intervention.) Note that t_{max} for insulin lispro for this subject was markedly higher than the mean (130 minutes in this experiment).

Table 1S. Baseline characteristics of subjects.

	All Subjects[†]
Number	6
Sex	3 M / 3 F
Age (years)	52 ± 14 (33–72)
BM (kg)	72 ± 10 (54–85)
BMI (kg/m^2)	25 ± 3 (22–30)
Diabetes duration (years)	32 ± 14 (17–50)
HbA1c (%)	7.4 ± 0.7 (6.4–8.3)
Daily insulin dose (units/kg)	0.45 ± 0.09 (0.31–0.56)
Stimulated c-peptide[‡] (nmol/L)	< 0.1

[†]Each subject contributed two 48-hour closed-loop visits.

[‡]All subjects had undetectable fasting and stimulated C-peptide, reported as less than the assay limit.