Supplementary Material for

Autonomous and Continuous Adaptation of a Bihormonal Bionic Endocrine Pancreas in Adults and Children with Type 1 Diabetes

This PDF file includes:

METHODS

SOM Note 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 application #G100062, approved by the Center for Devices and Radiological Health. We received an Investigational New Drug Exemption from the FDA for the use for glucagon in a pump for up to 27 hours.

SOM Note 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.

SOM Note 3: Closed-loop glucose control system

The insulin control algorithms used in the present study were built around the Model Predictive Control (MPC) algorithm used in our previous studies (*1, 2*). The insulin control algorithms used in the present study include several significant modifications. Firstly, the basal insulin algorithm was revised to allow the basal infusion rate of insulin to adapt online and to vary on multiple time scales. Secondly, a meal-priming insulin algorithm was developed, which automatically adapts the magnitude of the meal-priming bolus based on regulation and dosing around past meals. Finally, the MPC algorithm itself was revised such that the aggressiveness that controls the magnitude of the insulin bolus doses was allowed to adapt online.

In the glucagon control algorithm, the dynamic formulation of the proportional and derivative gains remained the same as in our previous studies, but the gain magnitudes were increased by $\sim 50\%$. This refinement to the glucagon algorithm was motivated by our analysis of hypoglycemic episodes that occurred during our previous studies (*1–3*).

SOM Note 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 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 manufacturers instruction 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 manufacturers instructions. In accordance with our Investigational New Drug 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. The commercially available

formulation of glucagon is unstable in solution (*4–6*). However, it has previously been shown in human experiments that the anti-hypoglycemic effect of this glucagon formulation given at microdoses is retained up to 27 hours after reconstitution in a pump reservoir without any apparent loss of efficacy (*1–3, 7*). In experiments performed in porcine models of diabetes, we and others have shown that the anti-hypoglycemic effects of this glucagon formulation are retained for up to seven days after reconstitution (*4, 8*). 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 (*4*). 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 (*4–6, 9*). Another Insulet OmniPod was filled with insulin lispro pod. A single insulin pod was used throughout each experiment except in cases of pod failure (noted in Supplementary Figures below). 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.

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 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 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 from 4:00 PM to approximately 4:30 PM.

During closed-loop control a CGMG measurement was streamed to a laptop computer residing on a modified IV pole via the Navigator cradle. 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. Blood samples for YSI verification of PG measurements and later insulin and glucagon measurement were drawn from a side port on the sampling line during the measurement phase of the GlucoScout cycle (approximately 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 planned meals provided the required calories for the subjects gender and age group to maintain energy balance at a sedentary activity level. These values are listed in Table S1. The macronutrient content of the diet was at least 50% carbohydrate, with no less than 33% of the total daily carbohydrates consumed in any one of the three daily meals. Maximum allowable carbohydrates for a single meal were 50% of the total estimated daily carbohydrate requirement (calculated as 0.5 $\times$ the calories required to maintain energy balance). This would allow subjects to eat up to 150% of the estimated carbohydrate requirement per day. To the extent possible, substitutions to the pre-planned menu were provided at the time of the experiment in the event that the subject decided to change their meal choice. The subjects was 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 so that the total calories and macronutrient content could be estimated and documented for each meal.

Each 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

Gender	Age (years)	Activity Level		
		Sedentary	Moderately Active	Active
Female	9–13	1,600	1,600–2,000	1,800–2,000
Female	14–18	1,800	2,000	2,400
Female	19–30	2,000	2,000–2,200	2,400
Female	31–50	1,800	2,000	2,200
Female	≥ 51	1,600	1,800	2,000–2,200
Male	9–13	1,800	1,800–2,200	2,000–2,600
Male	14–18	2,200	2,400–2,800	2,800–3,200
Male	19–30	2,400	2,600–2,800	3,000
Male	31–50	2,200	2,400–2,600	2,800–3,000
Male	≥ 51	2,000	2,200–2,400	2,400–2,800

Table S1. Estimated Calorie Requirements (in kilocalories) for Each Gender and Age Group at Three Levels of Physical Activity. These levels are based on Estimated Energy Requirements (EER) from the IOM Dietary Reference Intakes macronutrients report (2002) calculated by gender, age, and activity level for reference-sized individuals. "Reference size," as determined by IOM, is based on median height and weight for ages up to age 18 years and median height and weight for that height that gives a BMI of 21.5 for adult females and 22.5 for adult males. Source: HHS/USDA Dietary Guidelines for Americans (2005).

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.

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

SOM Note 5: Statistical analysis

The main outcome measures reported in the Results section and in the first row of the Table 2 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 2 shows the means and 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 SD may be different for the two different calculation methods because there may be different numbers of points from each subject.

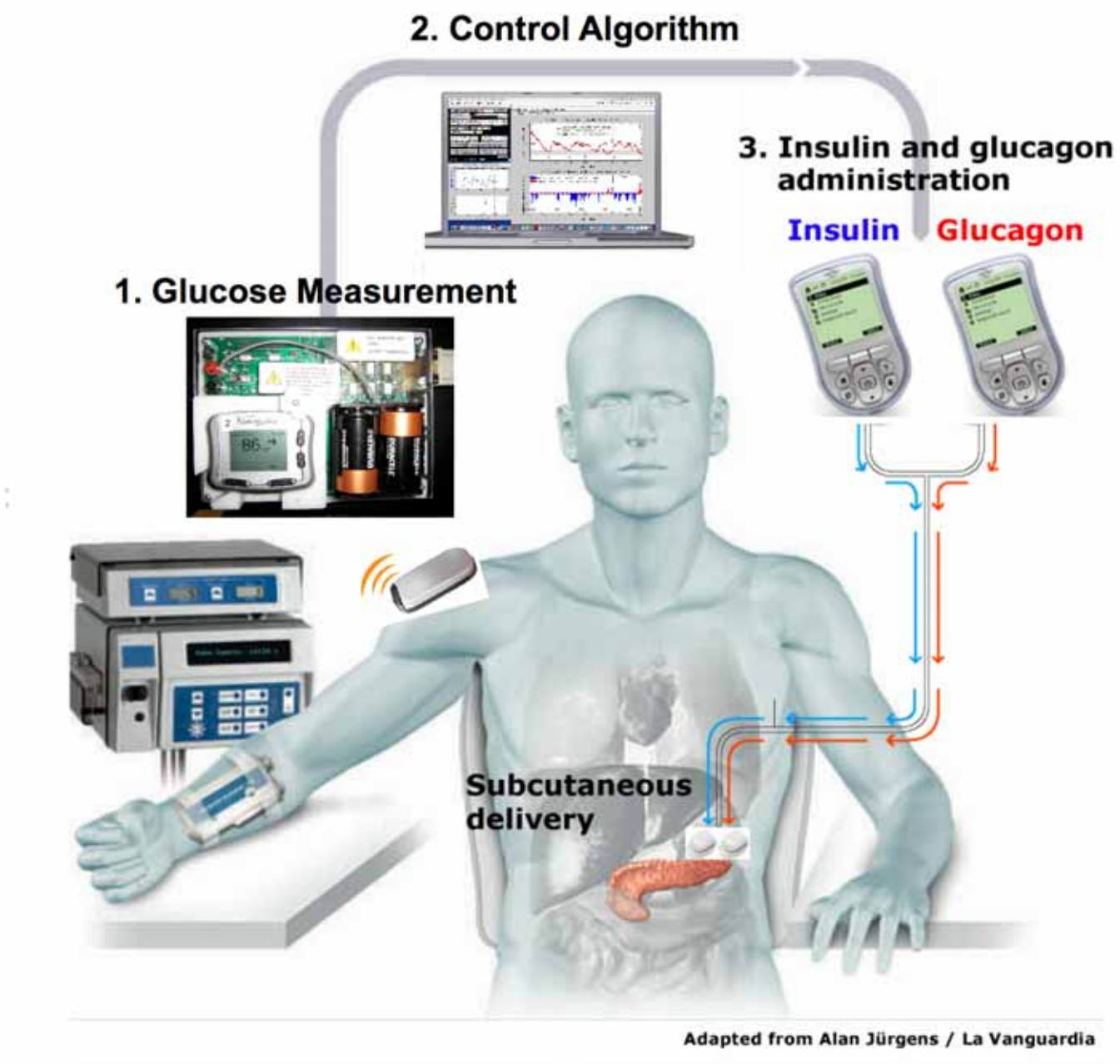

Fig. S1. Schematic of the automated bihormonal closed-loop control system used in the clinical trial. The controller responded to glucose readings streamed online every five minutes from a Navigator CGM (Abbott Diabetes Care), and commanded insulin–glucagon control doses wirelessly using the OmniPod infusion system (Insulet). Venous PG was also measured every 15 minutes using an FDA-approved GlucoScout (International Biomedical).

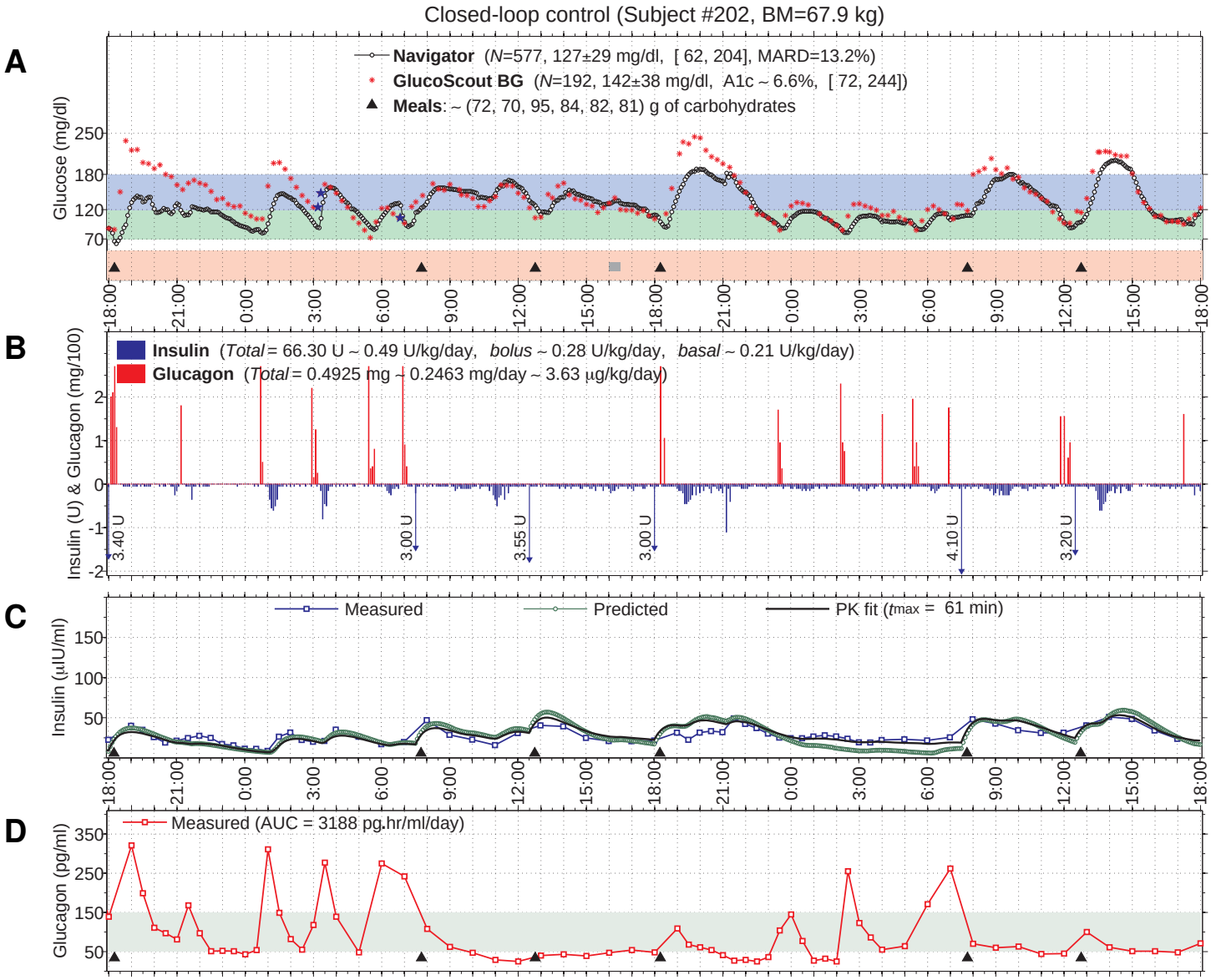

Fig. S2. 48-hour closed-loop experiment in adult female #202 with adaptive meal priming bolus (AMB). Respective means in CGMG and PG were 125 mg/dl and 142 mg/dl in the first 24-hour period (mean absolute relative difference [MARD] of 16%), and 128 mg/dl and 143 mg/dl in the second 24-hour period (MARD of 10%), with respective total insulin and glucagon doses being 0.43 U/kg and 0.25 mg in the first 24-hour period, and 0.55 U/kg and 0.24 mg in the second 24-hour period. In the first nine hours of this experiment CGMG (which is the sole online input to the algorithm) was underestimating PG substantially, which resulted in very little insulin dosing beyond basal insulin and the first meal priming dose, despite reference PG values that rose to $>$ 200 mg/dl. The disparity between CGMG and PG was only corrected later by the calibration routinely requested by the Navigator just past 3:00 on the first night. The experimental procedure was explicitly designed to avoid overcorrecting erroneous CGMG signals except at regularly scheduled calibration times or at regularly scheduled confirmatory check times (which were performed daily at 6:30 and 17:00), 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. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. The exercise session was stopped early during this experiment (at roughly 2900 heart beats instead of the planned 4000 heart beats) due to subject complaints of fatigue and nausea. The subject was noted to be pale with cool skin, and had a sudden decrease in heart rate from 120 to 60 bpm. She was taken off of the exercise bicycle and vomited a small amount. Symptoms resolved within minutes and did not appear to be correlated with PG or glucagon levels; the most recent glucagon dose was given more than nine hours prior to the episode. This was suspected to be a vasovagal episode.

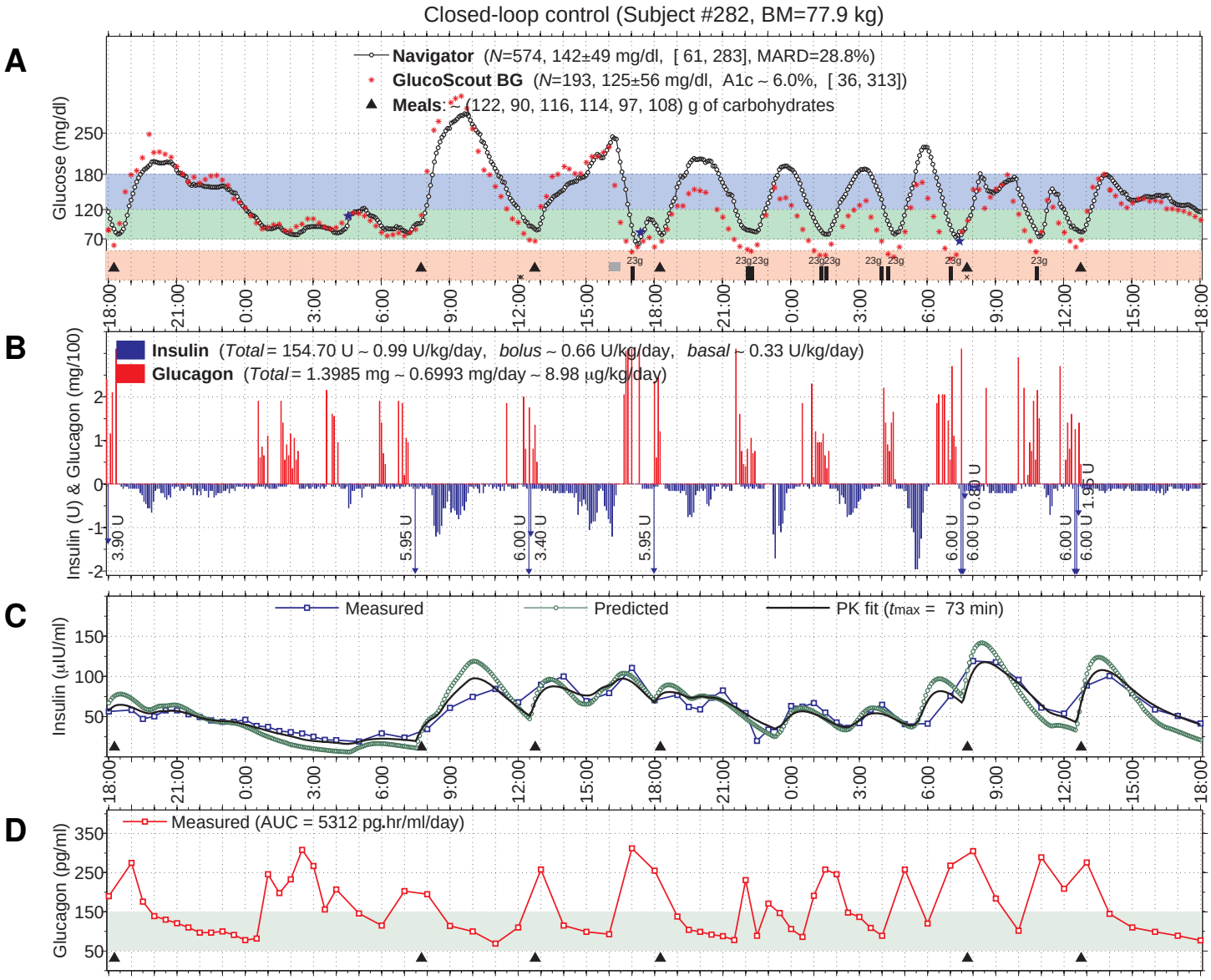

Fig. S3. 48-hour closed-loop experiment in adult male #282 with AMB. Respective means in CGMG and PG were 144 mg/dl and 142 mg/dl in the first 24-hour period (MARD of 17%), and 139 mg/dl and 107 mg/dl in the second 24-hour period (MARD of 41%), with respective total insulin and glucagon doses being 0.91 U/kg and 0.63 mg in the first 24-hour period, and 1.08 U/kg and 0.79 mg in the second 24-hour period. There was one hypoglycemic episode (venous PG $<$ 60 mg/dl) in the first 24-hour period (indicated by small black rectangle along the timeline of Panel A and annotated with the carbohydrate content of each intervention), which occurred simultaneously with a glucagon pod failure. The glucagon pod began alarming at 17:00, a hypoglycemic intervention was administered, and the glucagon pod was replaced. Five separate hypoglycemic episodes occurred in the second 24-hour period in this experiment. Over much of this same period, the CGMG overestimated PG, with a high MARD of 41%. Many or all of these hypoglycemic episodes in the second 24-hour period might have been prevented by re-calibrating the Navigator. In real-world use, a patient could re-calibrate the device after experiencing hypoglycemia and noticing a large discrepancy between the CGMG and PG levels.

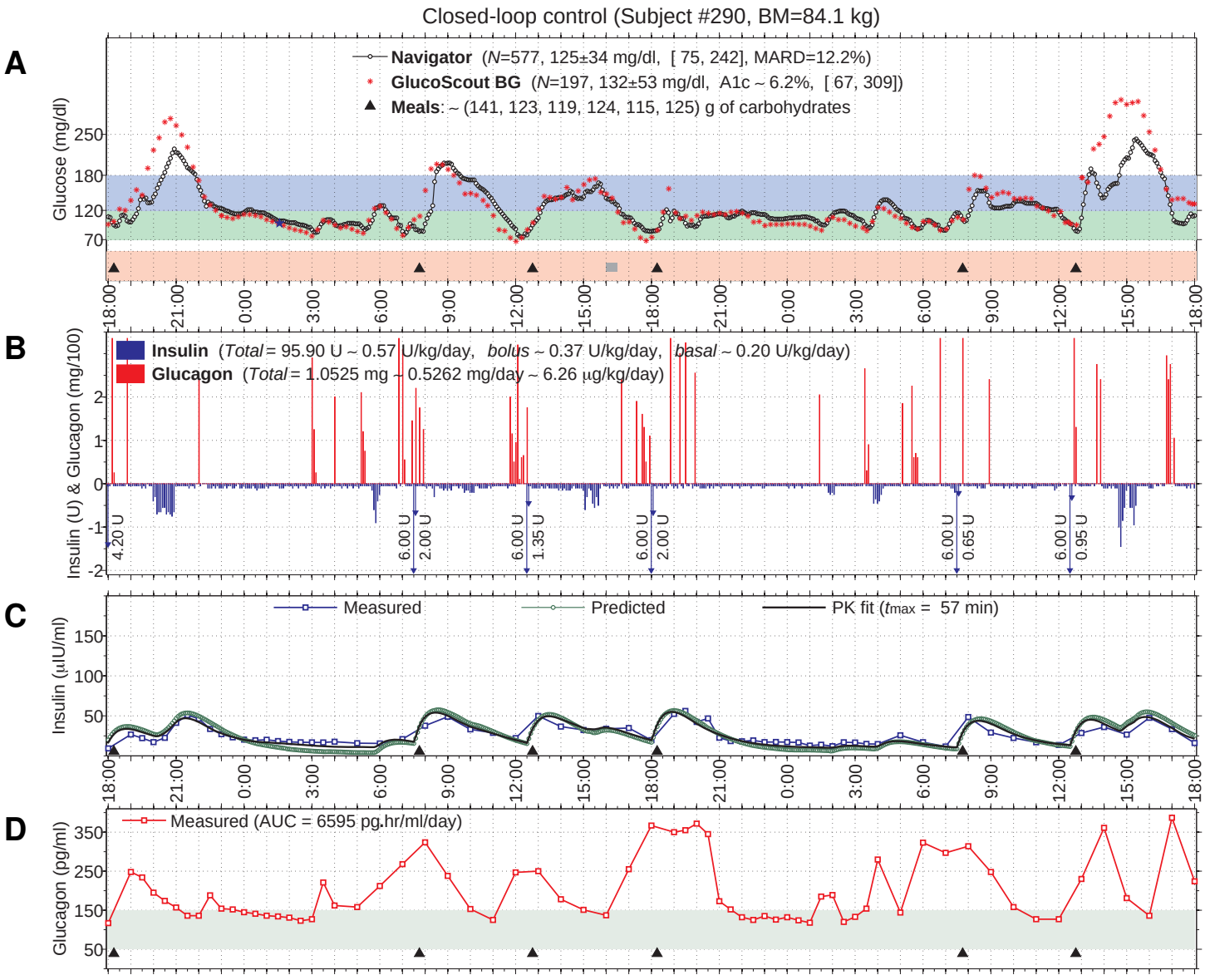

Fig. S4. 48-hour closed-loop experiment in adult male #290 with AMB. Respective means in CGMG and PG were 126 mg/dl and 129 mg/dl in the first 24-hour period (MARD of 12%), and 124 mg/dl and 135 mg/dl in the second 24-hour period (MARD of 12%), with respective total insulin and glucagon doses being 0.60 U/kg and 0.53 mg in the first 24-hour period, and 0.54 U/kg and 0.52 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

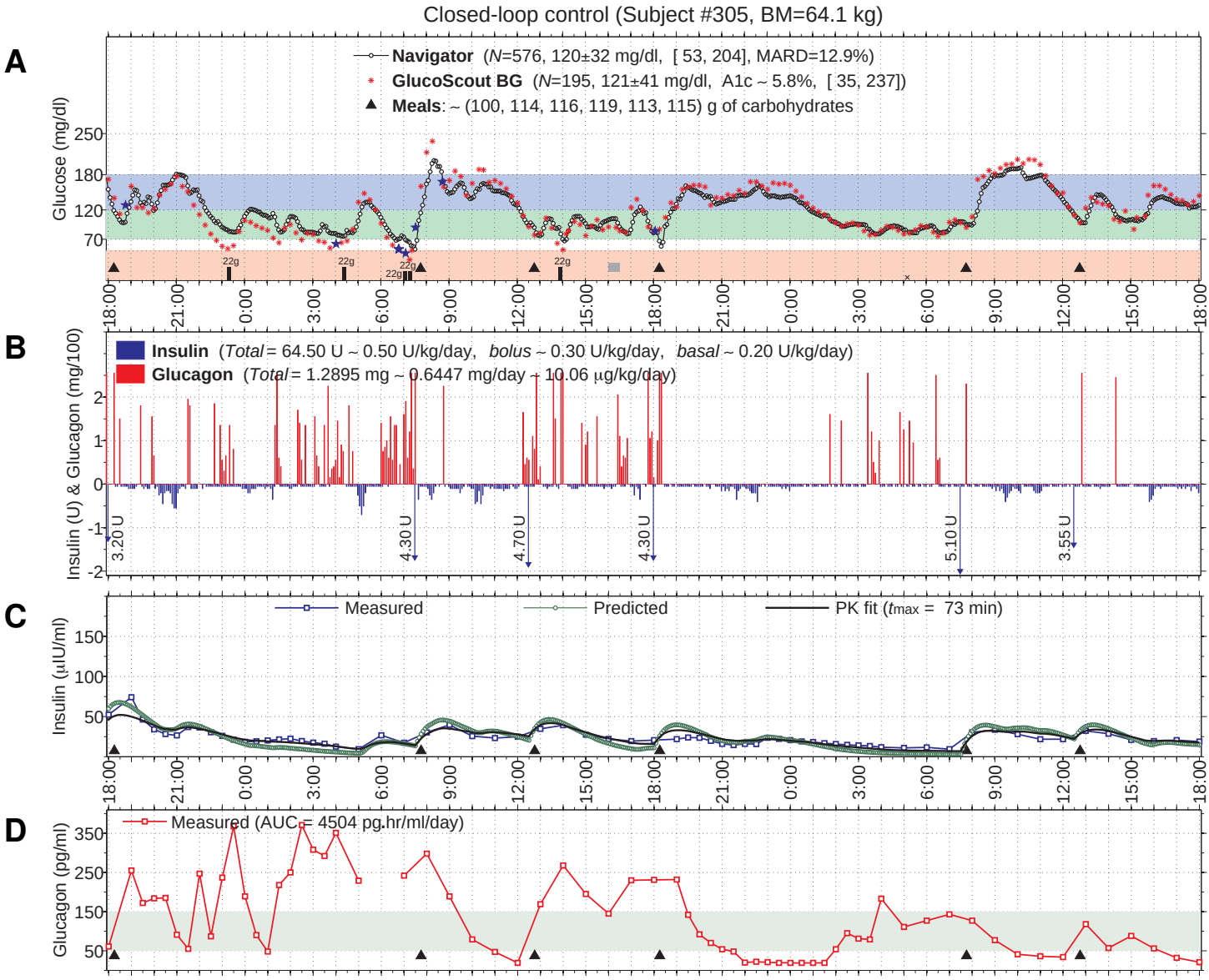

Fig. S5. 48-hour closed-loop experiment in adult male #305 with AMB. Respective means in CGMG and PG were 115 mg/dl and 110 mg/dl in the first 24-hour period (MARD of 18%), and 125 mg/dl and 132 mg/dl in the second 24-hour period (MARD of 8%), with respective total insulin and glucagon doses being 0.53 U/kg and 0.98 mg in the first 24-hour period, and 0.47 U/kg and 0.31 mg in the second 24-hour period. Hypoglycemic episodes (venous PG $<$ 60 mg/dl) occurred on four different occasions in the first 24-hour period (indicated by small black rectangles along the timeline of Panel A and annotated with the carbohydrate content of each intervention). Calibration of the Navigator occurred during a rapid rise in PG at 18:45. Immediately after this calibration, and throughout the first overnight period, the CGMG overestimated PG. This may have contributed to three of the hypoglycemic episodes that occurred before the first breakfast meal. Additionally, one of these hypoglycemic episodes (PG of 64 mg/dl at 04:15) was treated with carbohydrate despite not meeting the criteria for carbohydrate intervention. In contrast, there were no hypoglycemic episodes in the second 24-hour period in this experiment, which may in part be due to the improved MARD and downward adaptation of aggressiveness in the insulin bolus algorithm relative to the first 24-hour period. The less aggressive insulin bolus algorithm may have also led to the reduced glucagon dosing in the second 24-hour period relative to the first (as can be seen in panel **B**), which in turn led to relatively reduced plasma glucagon levels (as can be seen in panel **D**). There were two glucagon pod failures during this experiment. In both instances, the pods began alarming and required replacement. Glucagon pods were replaced at 18:00 on Day 1 and again at 11:30 on Day 2.

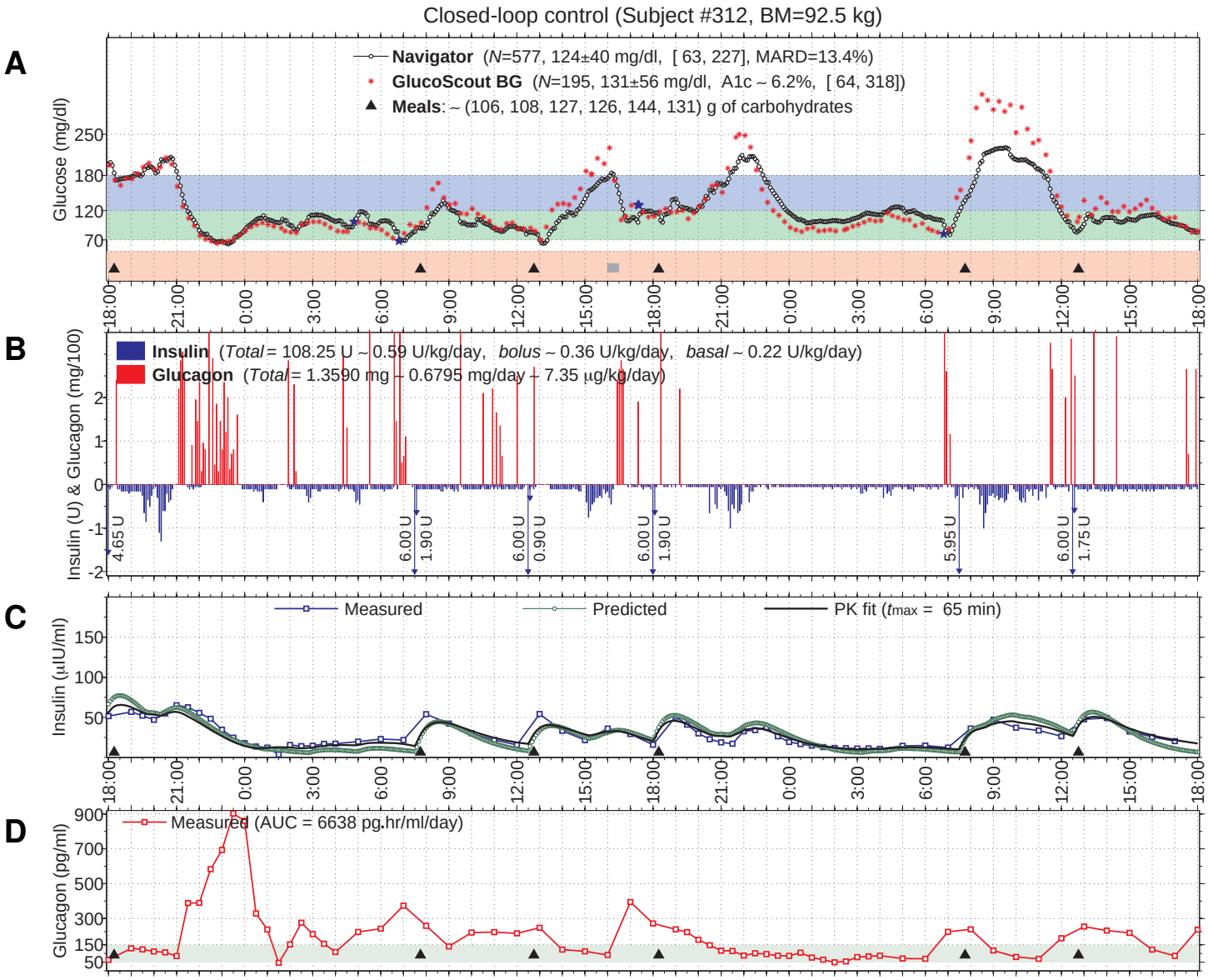

Fig. S6. 48-hour closed-loop experiment in adult male #312 with AMB. Respective means in CGMG and PG were 115 mg/dl and 119 mg/dl in the first 24-hour period (MARD of 11%), and 132 mg/dl and 144 mg/dl in the second 24-hour period (MARD of 16%), with respective total insulin and glucagon doses being 0.57 U/kg and 0.96 mg in the first 24-hour period, and 0.60 U/kg and 0.40 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. There were two glucagon pod failures during this experiment. In both instances, the pods began alarming and required replacement. Glucagon pods were replaced at 12:30 and 12:49 on Day 2. The meal was delayed by 20 minutes during pod replacement despite the meal priming bolus being given on schedule at 12:30. The PG dropped to 70 mg/dl at which point the subject began eating.

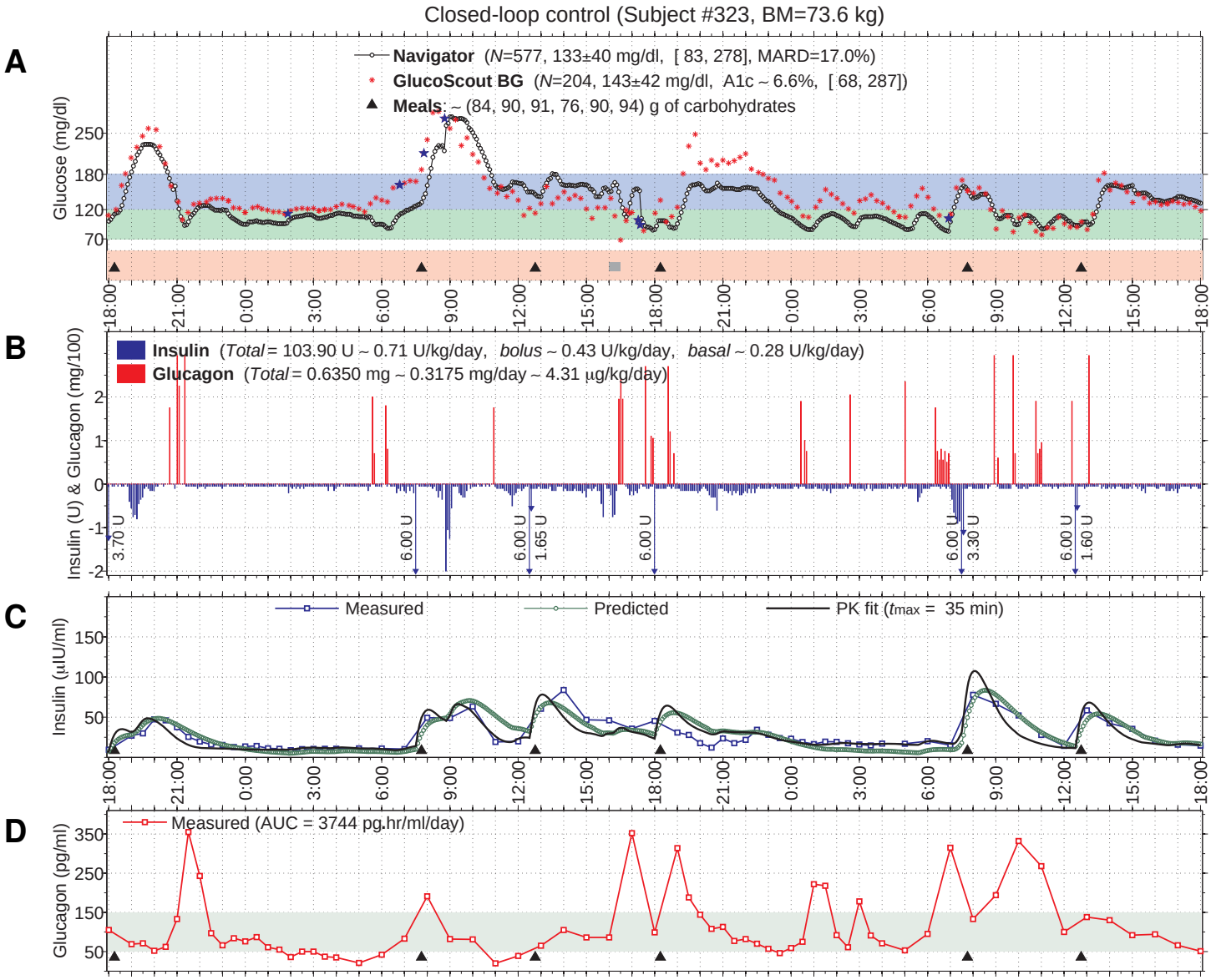

Fig. S7. 48-hour closed-loop experiment in adult female #323 with AMB. Respective means in CGMG and PG were 145 mg/dl and 148 mg/dl in the first 24-hour period (MARD of 18%), and 121 mg/dl and 138 mg/dl in the second 24-hour period (MARD of 16%), with respective total insulin and glucagon doses being 0.68 U/kg and 0.28 mg in the first 24-hour period, and 0.73 U/kg and 0.35 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

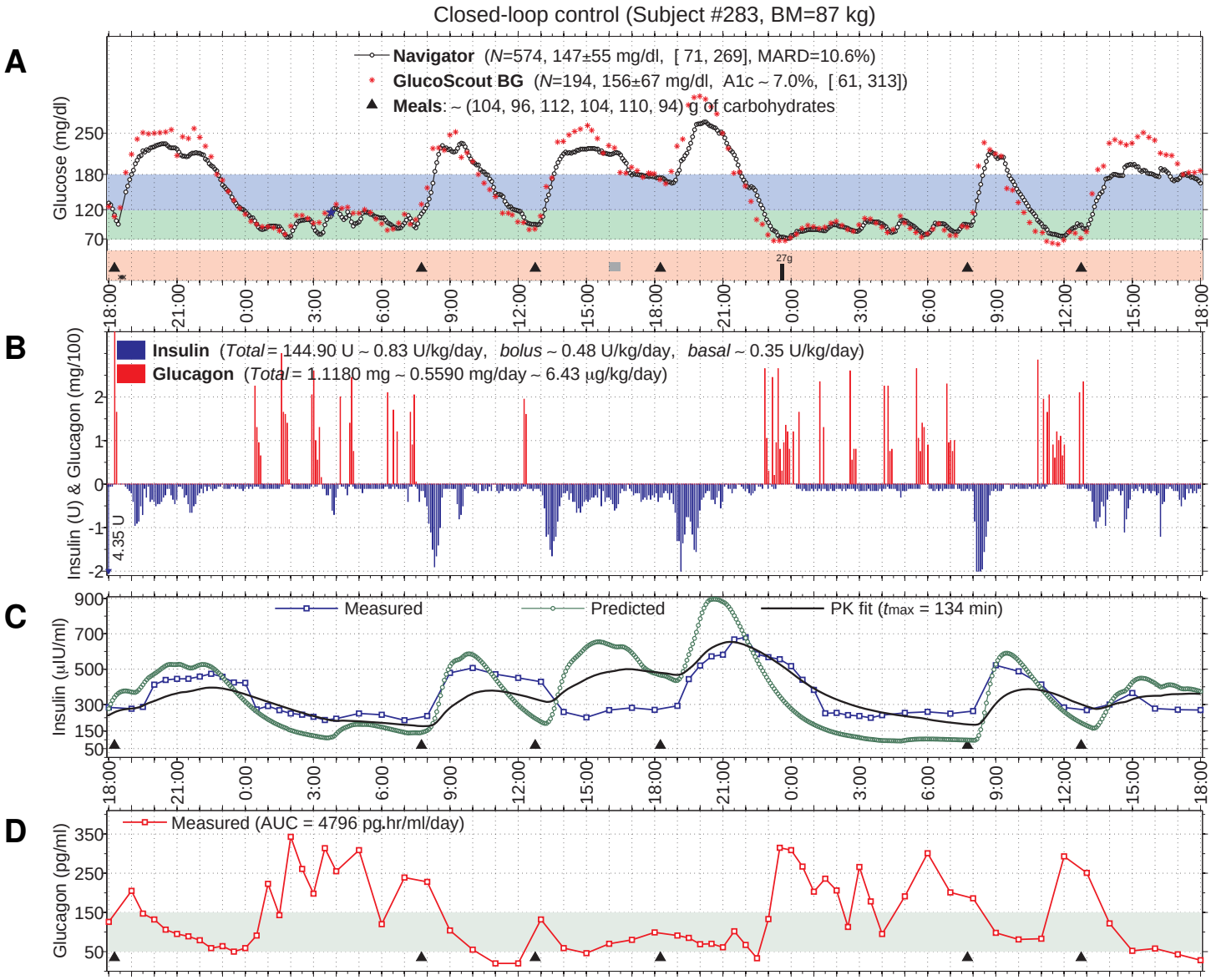

Fig. S8. 48-hour closed-loop experiment in adult male #283 with no meal priming bolus (NMB). Respective means in CGMG and PG were 156 mg/dl and 164 mg/dl in the first 24-hour period (MARD of 9%), and 138 mg/dl and 147 mg/dl in the second 24-hour period (MARD of 12%), with respective total insulin and glucagon doses being 0.86 U/kg and 0.46 mg in the first 24-hour period, and 0.81 U/kg and 0.66 mg in the second 24-hour period. A meal priming bolus of 4.35 units of insulin was inadvertently given at 18:00 on Day 1. Since this subject was randomized into the "no meal priming bolus" arm, the adaptive bolus feature was turned off for the remainder of the experiment. This experiment was affected by an insulin delivery failure sometime around 13:00 on the Day 2. The insulin pod was replaced due to clinical suspicion at 15:20. The suspected insulin delivery failure was subsequently verified based on measured plasma insulin levels, which did not rise as expected in response to insulin administered following the 12:30 meal on Day 2. The missed insulin delivery is shown by the disparity between predicted insulin levels (green circles) and measured insulin levels (blue circles) from 13:00 to 19:30 on the Day 2. After pod replacement, the insulin levels began to rise in response to dosing after the second dinner. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment, but there was a single carbohydrate intervention $\sim$ 11:30 PM in the second 24-hour period. The subject complained of headaches and nausea on the second day at 06:15, 12:10, 23:30 and on the third day at 06:15 and 11:30. Each of these episodes were treated with Ibuprofen and symptoms resolved within two hours. In three of these episodes, the symptoms appear to be correlated with high levels of glucagon. The subject also shared that his level of caffeine intake was significantly lower during the study period, which may have contributed to his symptoms.

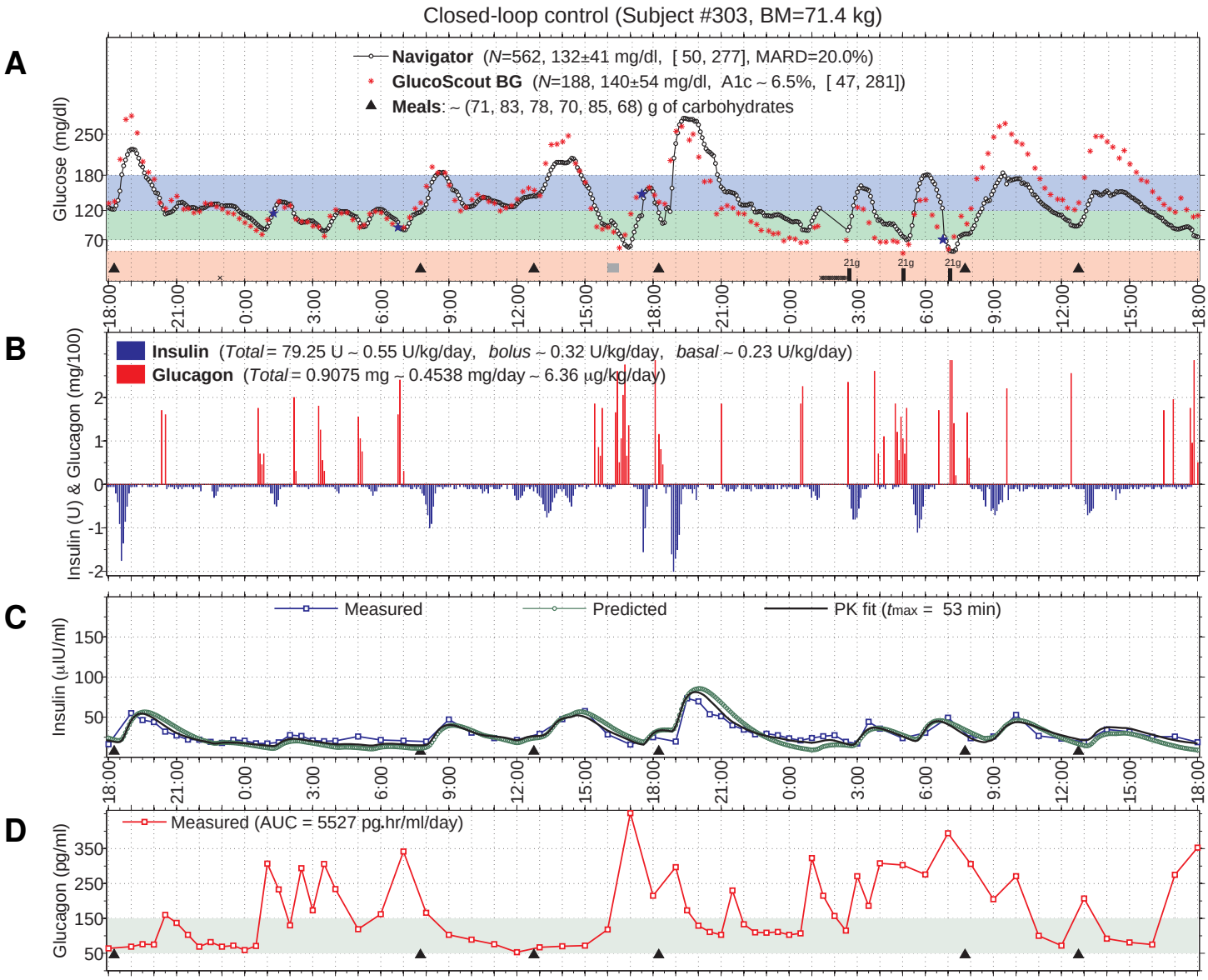

Fig. S9. 48-hour closed-loop experiment in adult female #303 with NMB. Respective means in CGMG and PG were 131 mg/dl and 136 mg/dl in the first 24-hour period (MARD of 10%), and 133 mg/dl and 144 mg/dl in the second 24-hour period (MARD of 31%), with respective total insulin and glucagon doses being 0.53 U/kg and 0.38 mg in the first 24-hour period, and 0.59 U/kg and 0.52 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in the first 24-hour period. There were three hypoglycemic episodes in the second 24-hour period. The Navigator stopped streaming CGMG on the third day from 01:45 until 02:15. The hypoglycemic episode at 02:15 occurred just as the device came back online. Hypoglycemic episodes occurred on the third day at 05:00 and 07:00. The glucagon pod began alarming and was replaced at 07:00, just prior to the third carbohydrate intervention. Over most of the first half of the second 24-hour period, the CGMG overestimated PG, with a high MARD of 31%. Many or all of these hypoglycemic episodes over this period might have been prevented by re-calibrating the Navigator. In real-world use, a patient could re-calibrate the device after experiencing hypoglycemia and noticing a large discrepancy between the CGMG and PG levels. The CGMG switched from significantly over-estimating PG to significantly underestimating PG after a calibration at 6:45 in the second 24-hour period.

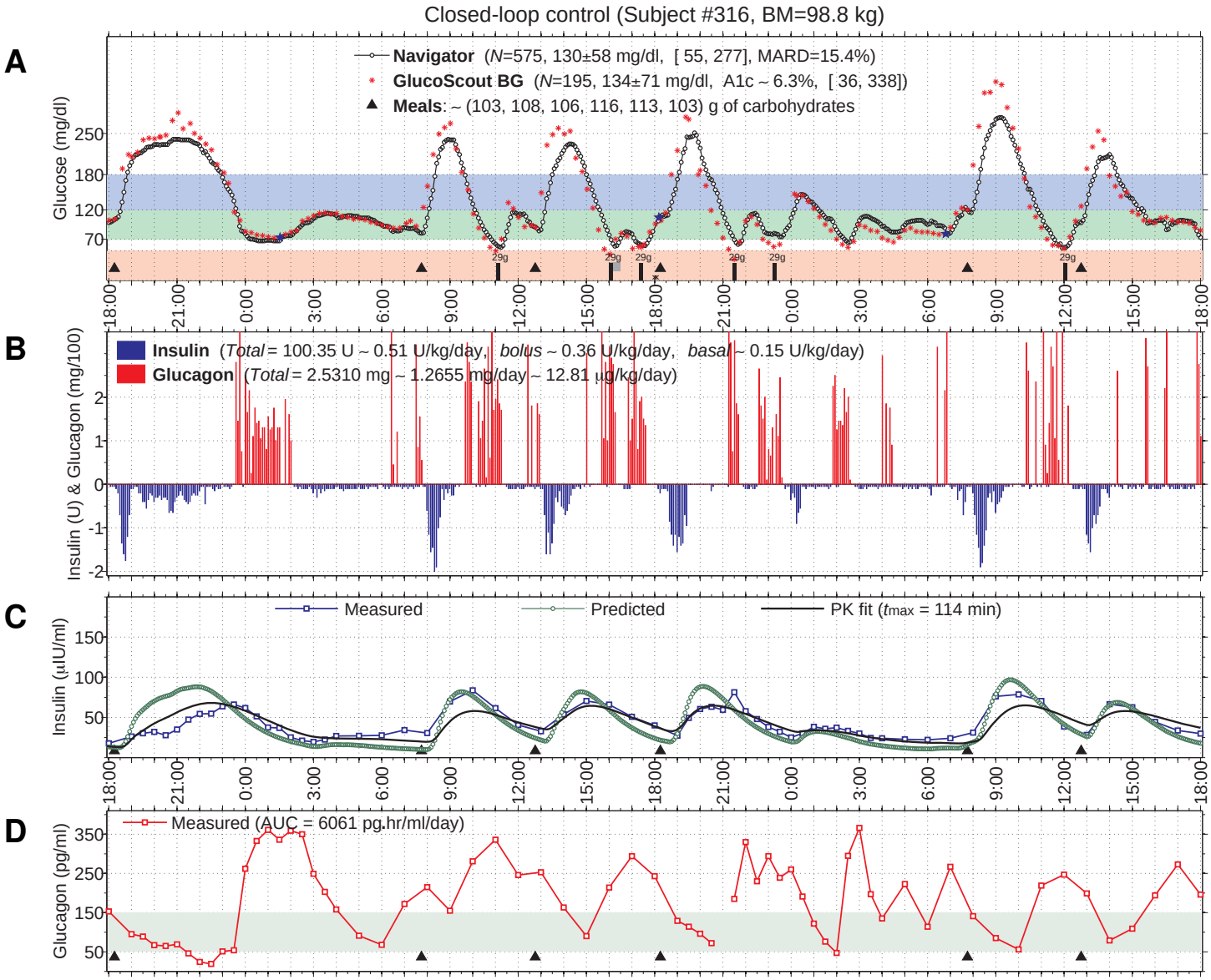

Fig. S10. 48-hour closed-loop experiment in adult male #316 with NMB. Respective means in CGMG and PG were 134 mg/dl and 142 mg/dl in the first 24-hour period (MARD of 13%), and 126 mg/dl and 127 mg/dl in the second 24-hour period (MARD of 18%), with respective total insulin and glucagon doses being 0.53 U/kg and 1.35 mg in the first 24-hour period, and 0.49 U/kg and 1.18 mg in the second 24-hour period. There were six unscheduled pod replacements during this experiment; five of these were identified as pod failures after they began alarming and required replacement. An insulin pod was replaced on the first day at 19:00. 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 22:00 on the first day. After pod replacement at 19:00, the insulin levels began to rise in response to dosing. A glucagon pod was replaced on the second day at 09:00, an insulin pod was replaced on the second day at 17:30 and again at 17:45, a glucagon pod was replaced on the second day at 21:15, and a glucagon pod was replaced on the third day at 12:00. There were three hypoglycemic episodes (venous PG $<$ 60 mg/dl) in each 24-hour period in this experiment. Note that two of the hypoglycemic episodes occurred immediately following glucagon pod failures on the second day at 21:15, and again on the third day at 12:00.

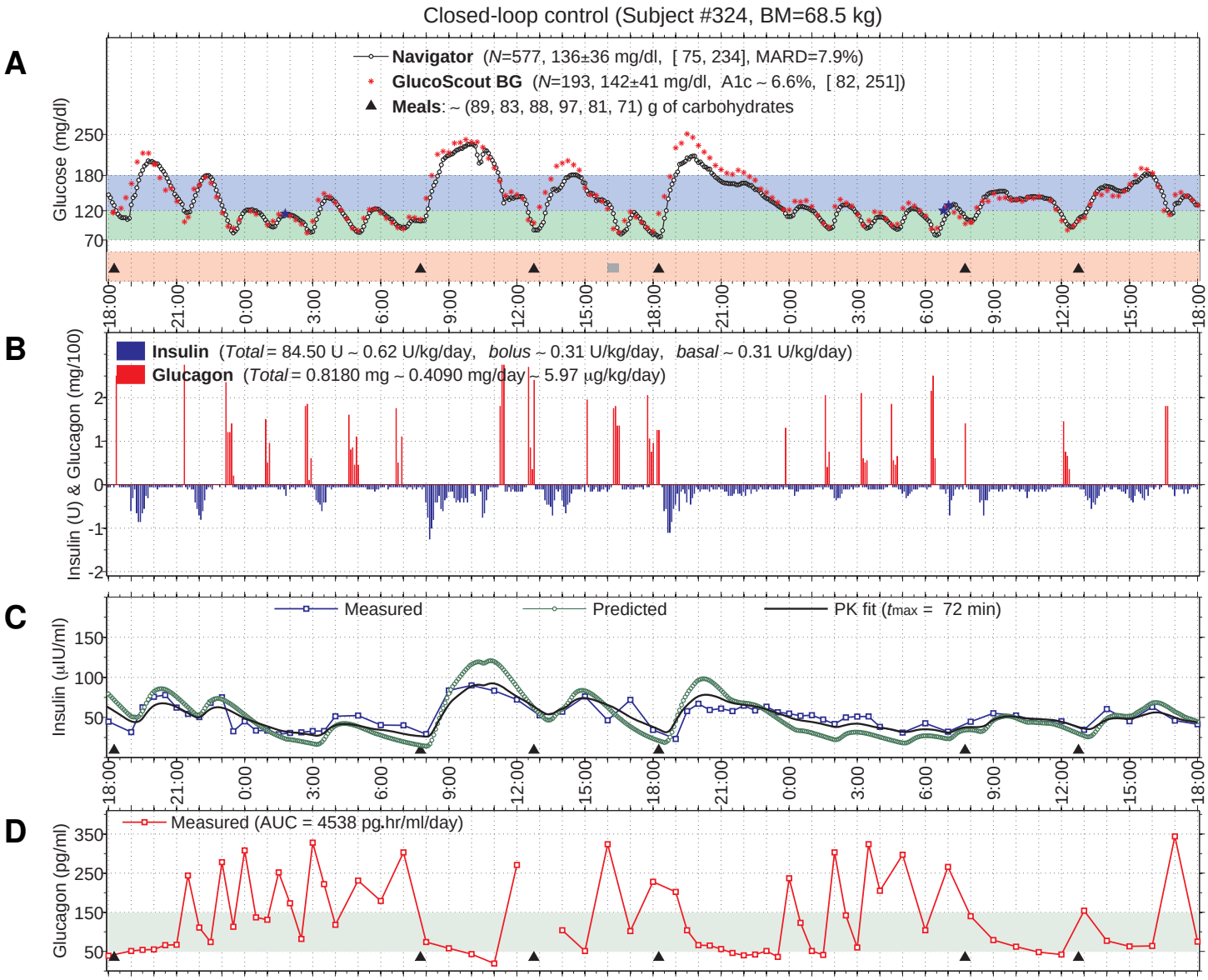

Fig. S11. 48-hour closed-loop experiment in adult female #324 with NMB. Respective means in CGMG and PG were 137 mg/dl and 143 mg/dl in the first 24-hour period (MARD of 8%), and 135 mg/dl and 141 mg/dl in the second 24-hour period (MARD of 7%), with respective total insulin and glucagon doses being 0.64 U/kg and 0.54 mg in the first 24-hour period, and 0.60 U/kg and 0.29 mg in the second 24-hour period. The subject complained of a headache at 14:30 on the third day. The headache resolved with Ibuprofen and did not appear to correlate with PG or plasma glucagon levels. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

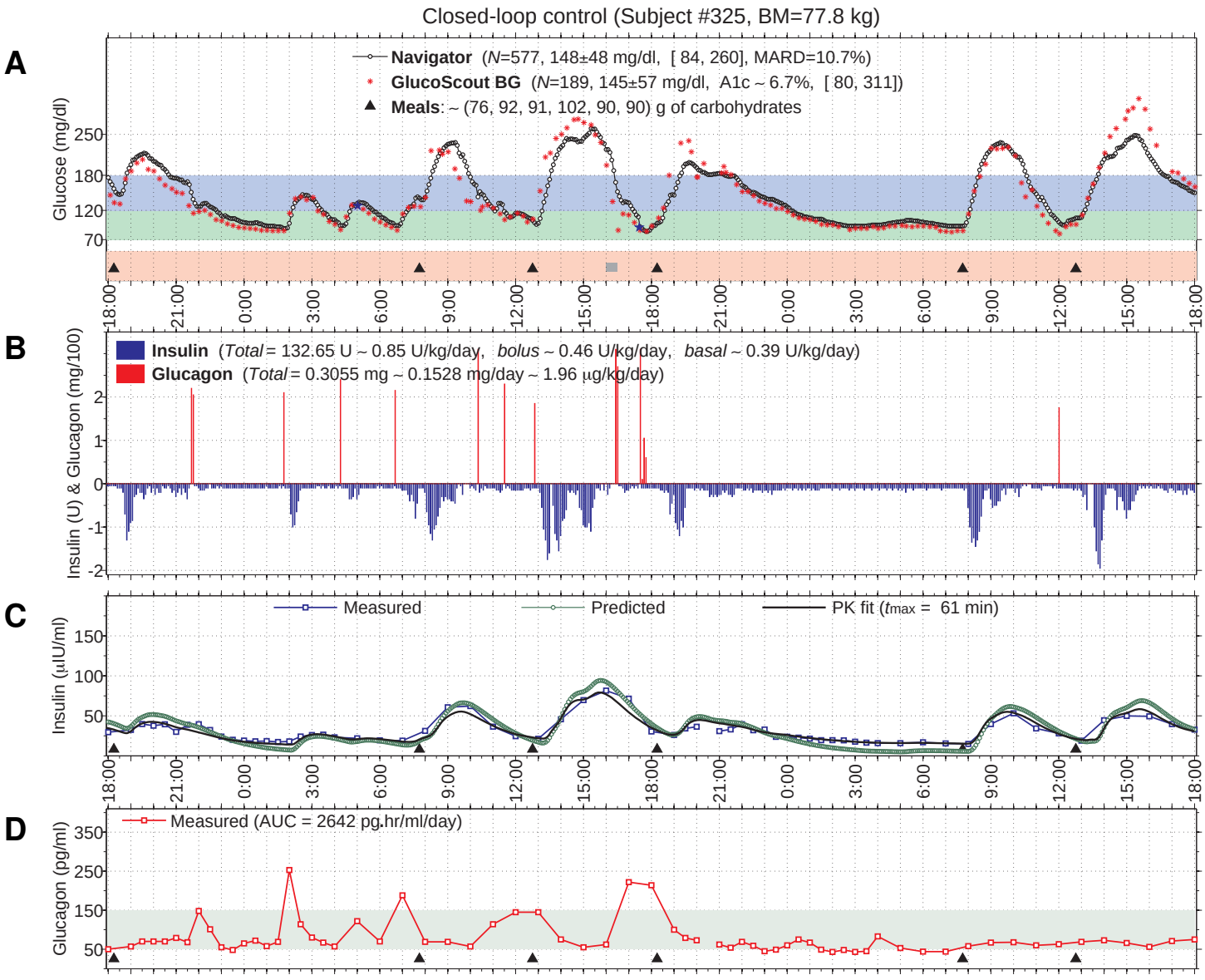

Fig. S12. 48-hour closed-loop experiment in adult female #325 with NMB. Respective means in CGMG and PG were 149 mg/dl and 141 mg/dl in the first 24-hour period (MARD of 12%), and 147 mg/dl and 149 mg/dl in the second 24-hour period (MARD of 9%), with respective total insulin and glucagon doses being 0.88 U/kg and 0.29 mg in the first 24-hour period, and 0.82 U/kg and 0.02 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

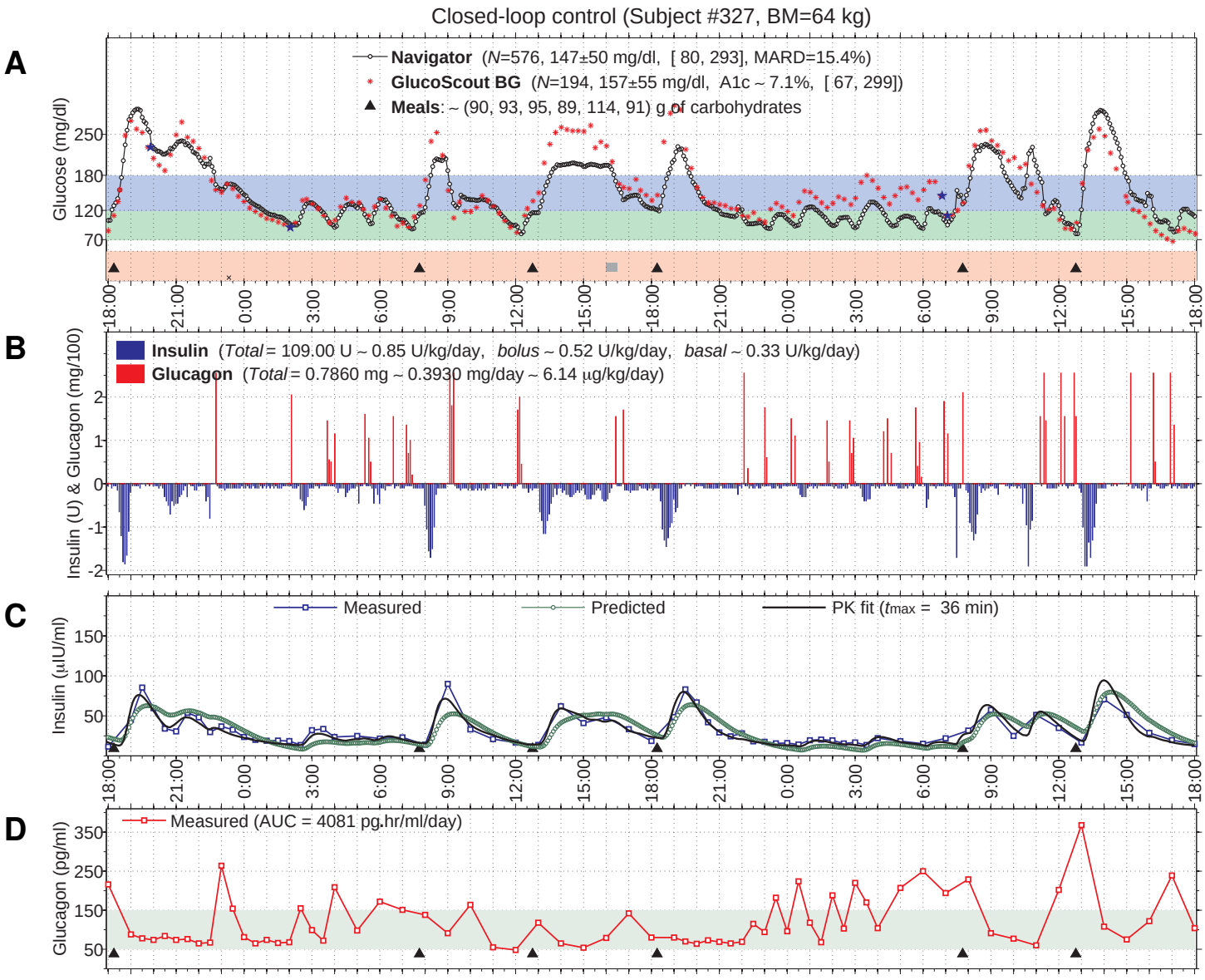

Fig. S13. 48-hour closed-loop experiment in adult female #327 with NMB. Respective means in CGMG and PG were 155 mg/dl and 162 mg/dl in the first 24-hour period (MARD of 11%), and 140 mg/dl and 152 mg/dl in the second 24-hour period (MARD of 20%), with respective total insulin and glucagon doses being 0.88 U/kg and 0.31 mg in the first 24-hour period, and 0.83 U/kg and 0.48 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

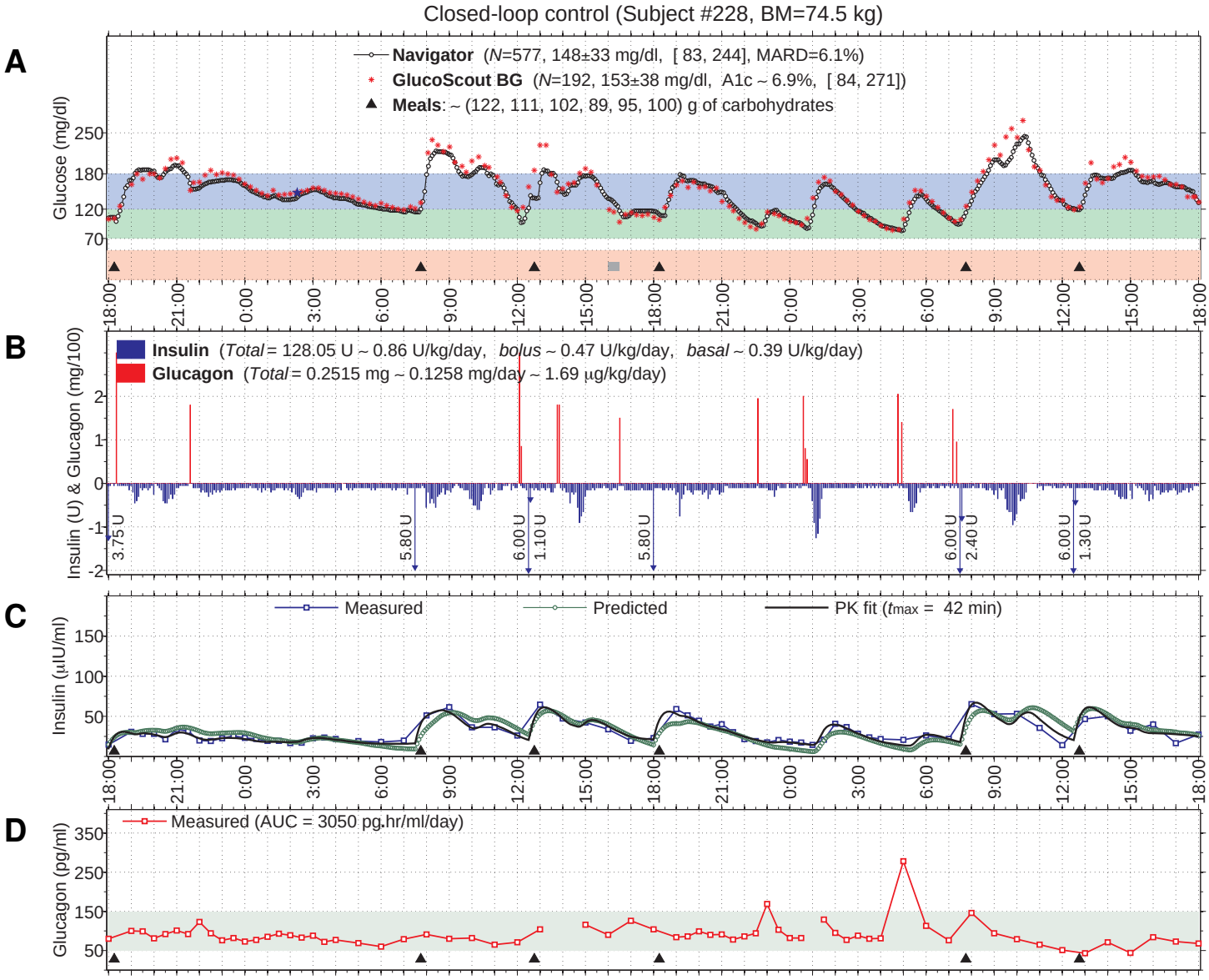

Fig. S14. 48-hour closed-loop experiment in pediatric female #228 with AMB. Respective means in CGMG and PG were 153 mg/dl and 159 mg/dl in the first 24-hour period (MARD of 6%), and 143 mg/dl and 146 mg/dl in the second 24-hour period (MARD of 6%), with respective total insulin and glucagon doses being 0.81 U/kg and 0.14 mg in the first 24-hour period, and 0.91 U/kg and 0.11 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. There was a suspected insulin pod failure on Day 3 at 10:00 after a rise in CGMG and PG values $>$ 200 mg/dl. Glucose levels subsequently declined after the insulin pod was replaced.

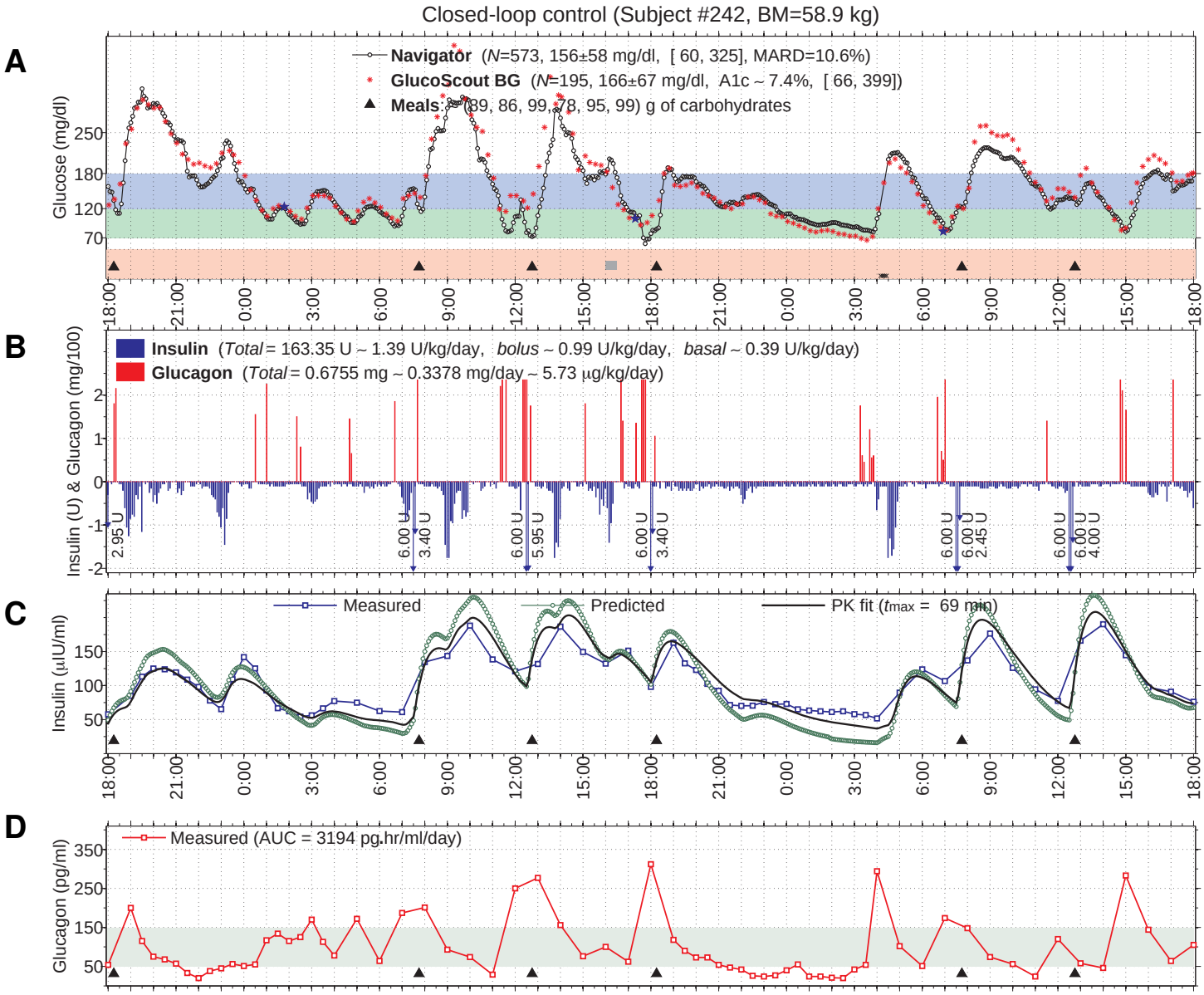

Fig. S15. 48-hour closed-loop experiment in pediatric female #242 with AMB. Respective means in CGMG and PG were 171 mg/dl and 187 mg/dl in the first 24-hour period (MARD of 11%), and 142 mg/dl and 146 mg/dl in the second 24-hour period (MARD of 10%), with respective total insulin and glucagon doses being 1.45 U/kg and 0.46 mg in the first 24-hour period, and 1.32 U/kg and 0.22 mg in the second 24-hour period. The subject did have a vasovagal episode related to a blood draw at 18:00 on day 1 of the experiment with any glucagon dosing and PG 134mg/dL at that time; however, there were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. The Navigator was offline on Day 3 for 15 minutes between 04:10 and 04:25. Controller-determined basal insulin dosing was delivered during that period.

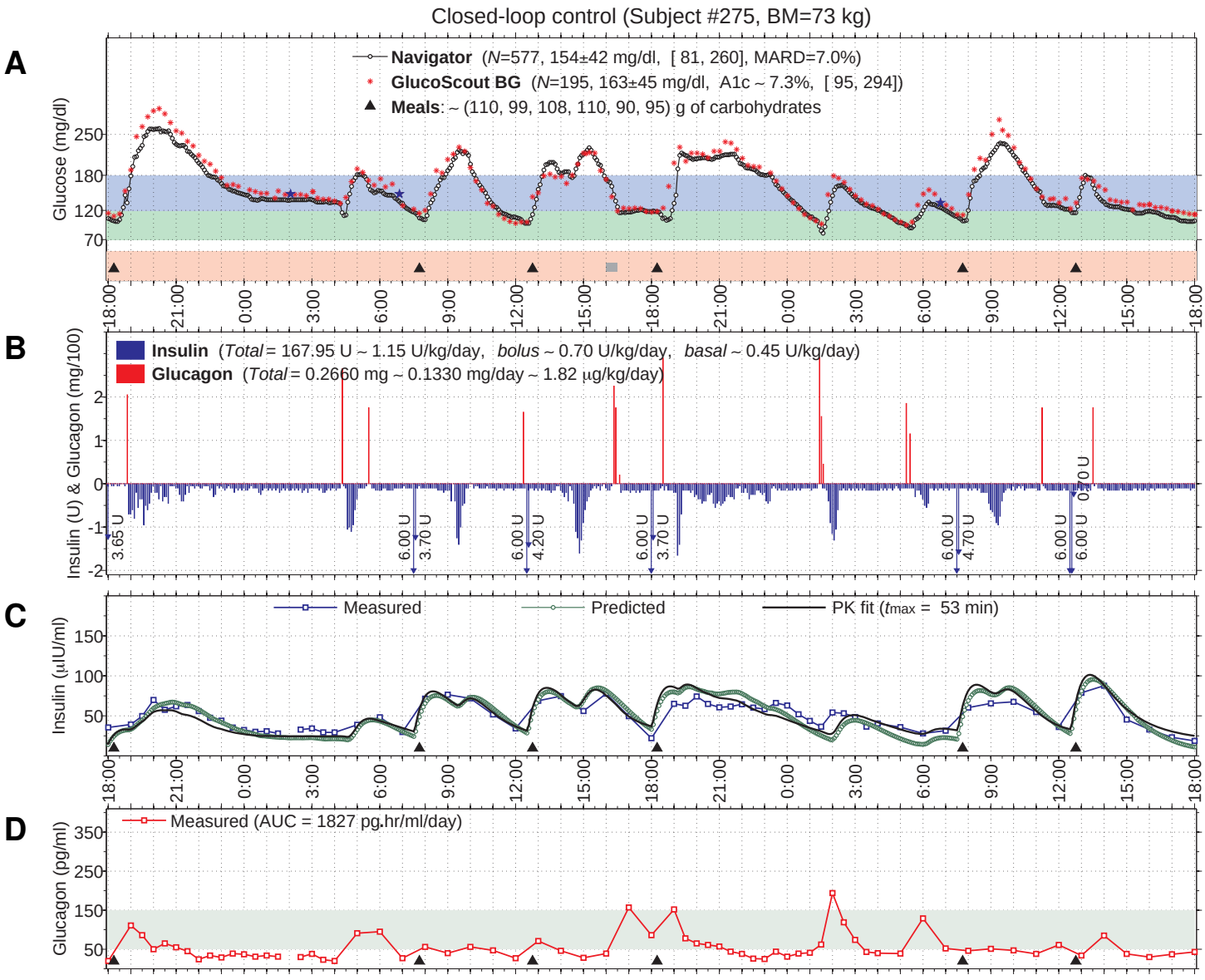

Fig. S16. 48-hour closed-loop experiment in pediatric female #275 with AMB. Respective means in CGMG and PG were 161 mg/dl and 169 mg/dl in the first 24-hour period (MARD of 7%), and 147 mg/dl and 156 mg/dl in the second 24-hour period (MARD of 7%), with respective total insulin and glucagon doses being 1.08 U/kg and 0.12 mg in the first 24-hour period, and 1.22 U/kg and 0.14 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

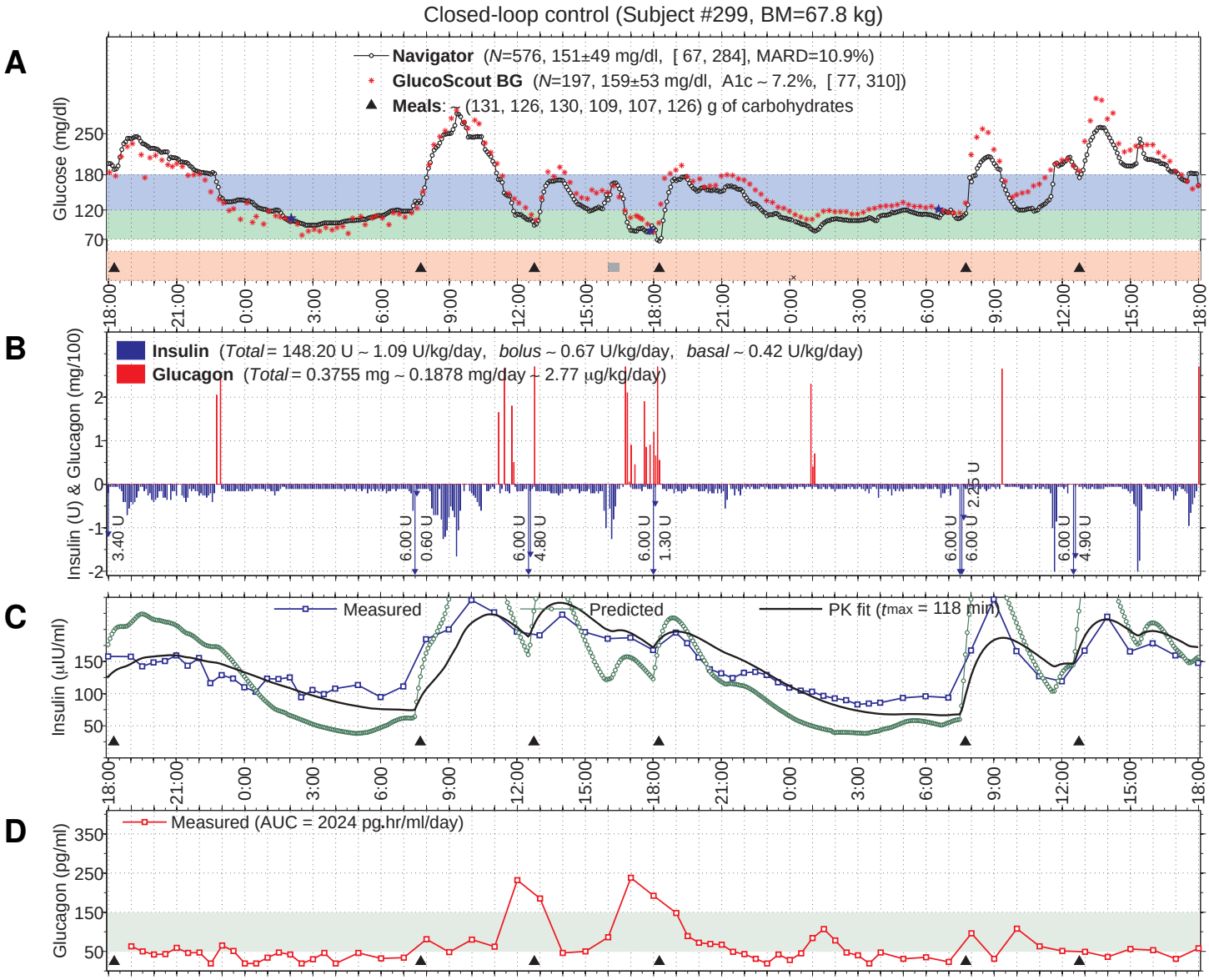

Fig. S17. 48-hour closed-loop experiment in pediatric male #299 with AMB. Respective means in CGMG and PG were 154 mg/dl and 151 mg/dl in the first 24-hour period (MARD of 10%), and 149 mg/dl and 167 mg/dl in the second 24-hour period (MARD of 12%), with respective total insulin and glucagon doses being 1.13 U/kg and 0.25 mg in the first 24-hour period, and 1.05 U/kg and 0.14 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. Calibration of the Navigator failed at 17:00 on Day 2. Subsequent to that, CGMG values underestimated PG values.

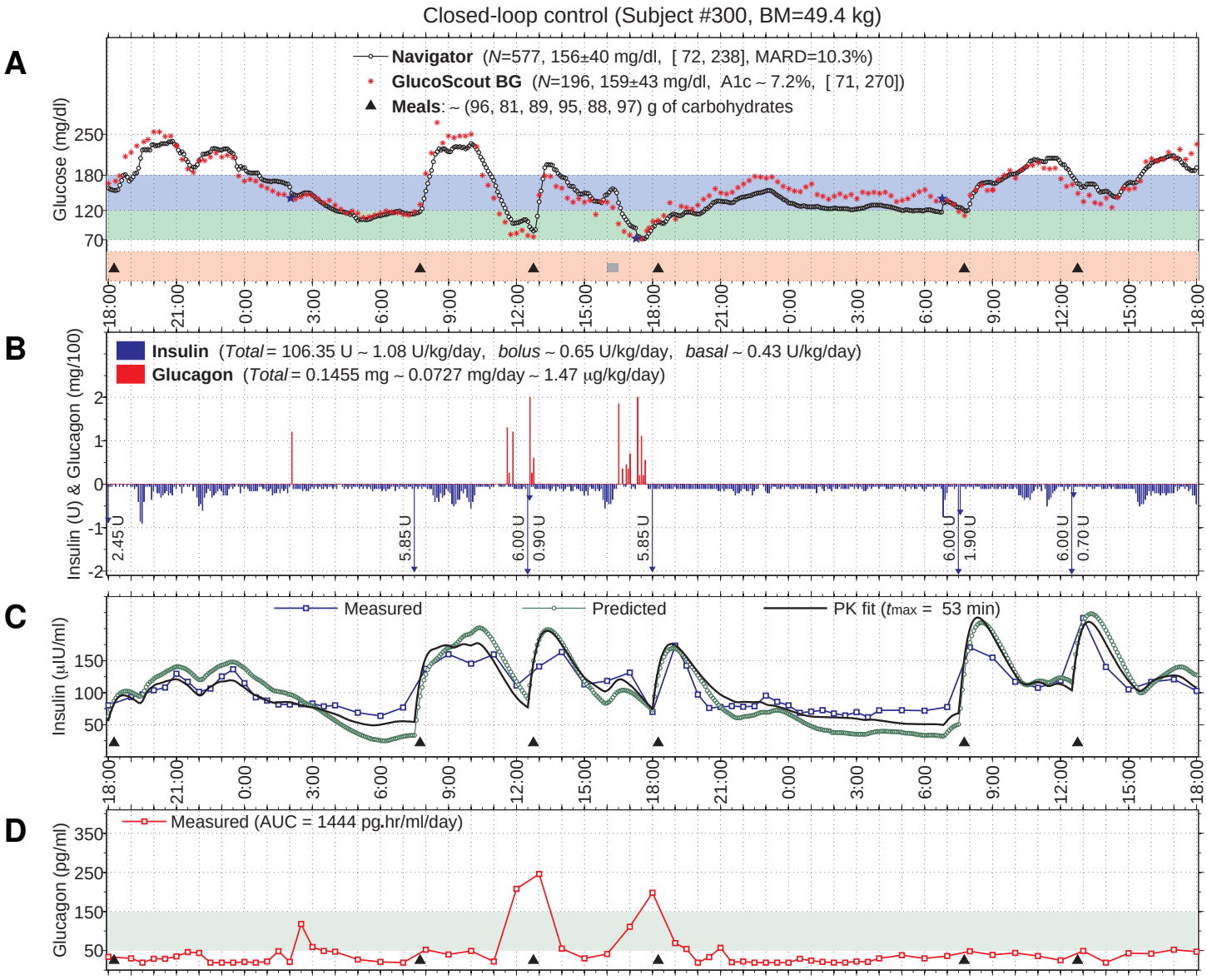

Fig. S18. 48-hour closed-loop experiment in pediatric female #300 with AMB. Respective means in CGMG and PG were 162 mg/dl and 159 mg/dl in the first 24-hour period (MARD of 10%), and 149 mg/dl and 158 mg/dl in the second 24-hour period (MARD of 11%), with respective total insulin and glucagon doses being 1.06 U/kg and 0.15 mg in the first 24-hour period, and 1.09 U/kg and 0 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. After calibration of the Navigator at 17:15 on Day 2, CGMG values were higher that PG values until 06:45 on Day 3, when it was noted to be out of range. A forced calibration was performed as per protocol at that time.

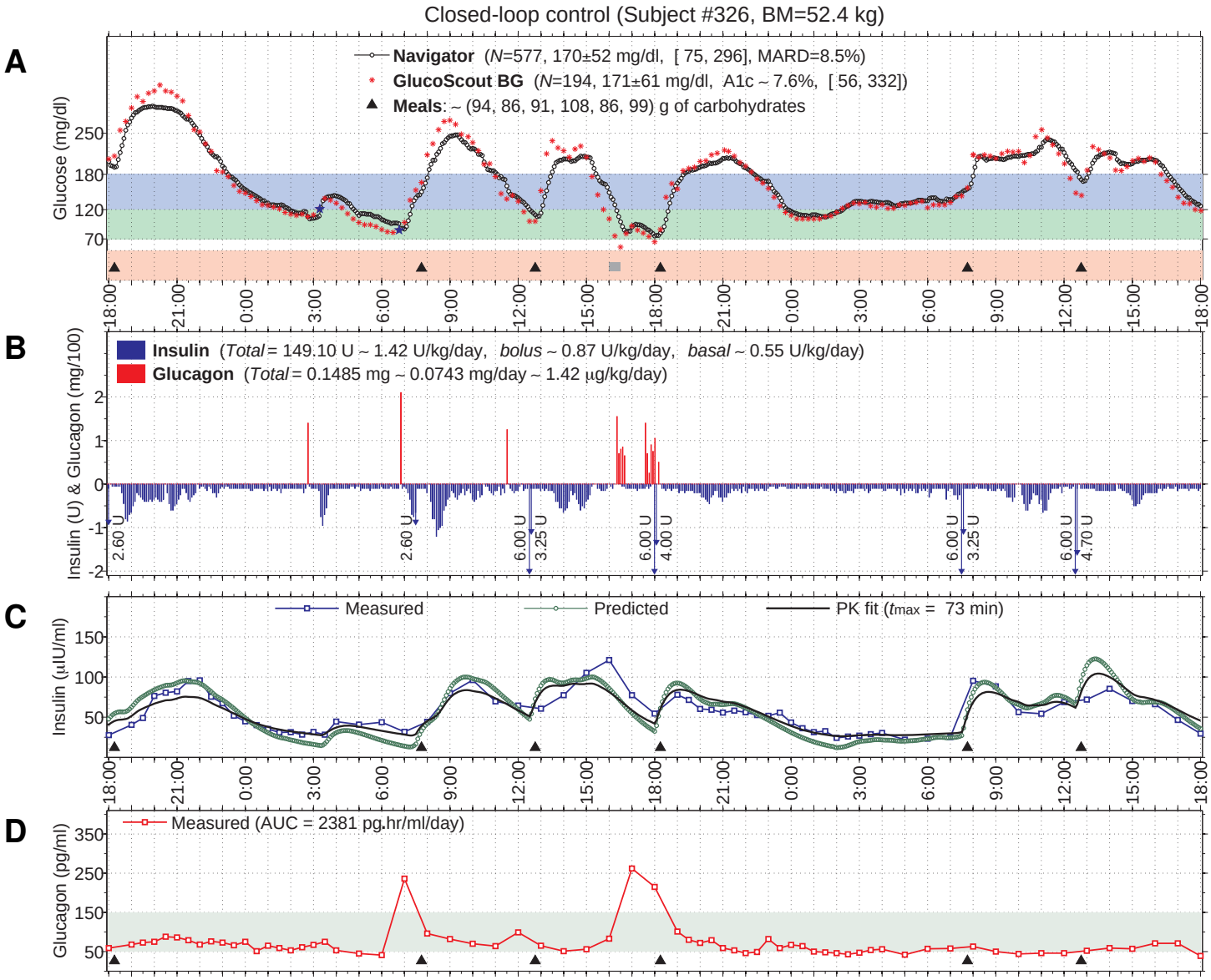

Fig. S19. 48-hour closed-loop experiment in pediatric female #326 with AMB. Respective means in CGMG and PG were 173 mg/dl and 174 mg/dl in the first 24-hour period (MARD of 11%), and 168 mg/dl and 166 mg/dl in the second 24-hour period (MARD of 6%), with respective total insulin and glucagon doses being 1.41 U/kg and 0.14 mg in the first 24-hour period, and 1.43 U/kg and 0.02 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

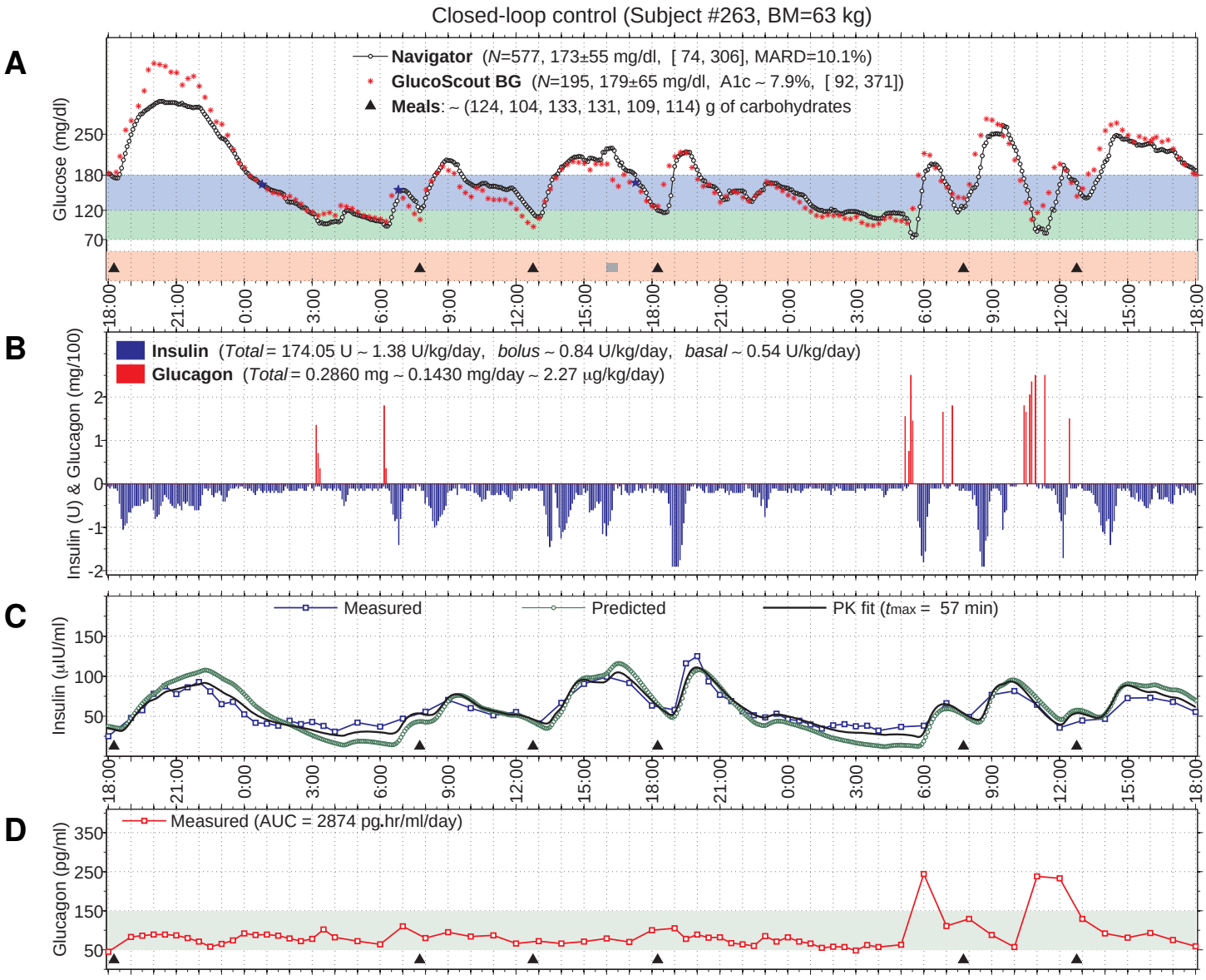

Fig. S20. 48-hour closed-loop experiment in pediatric male #263 with NMB. Respective means in CGMG and PG were 181 mg/dl and 184 mg/dl in the first 24-hour period (MARD of 9%), and 164 mg/dl and 173 mg/dl in the second 24-hour period (MARD of 11%), with respective total insulin and glucagon doses being 1.41 U/kg and 0.05 mg in the first 24-hour period, and 1.35 U/kg and 0.24 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. After prolonged hyperglycemia from 18:45 to 22:45 on Day 1, a betahydroxybutyrate level was obtained as per protocol, and returned negative.

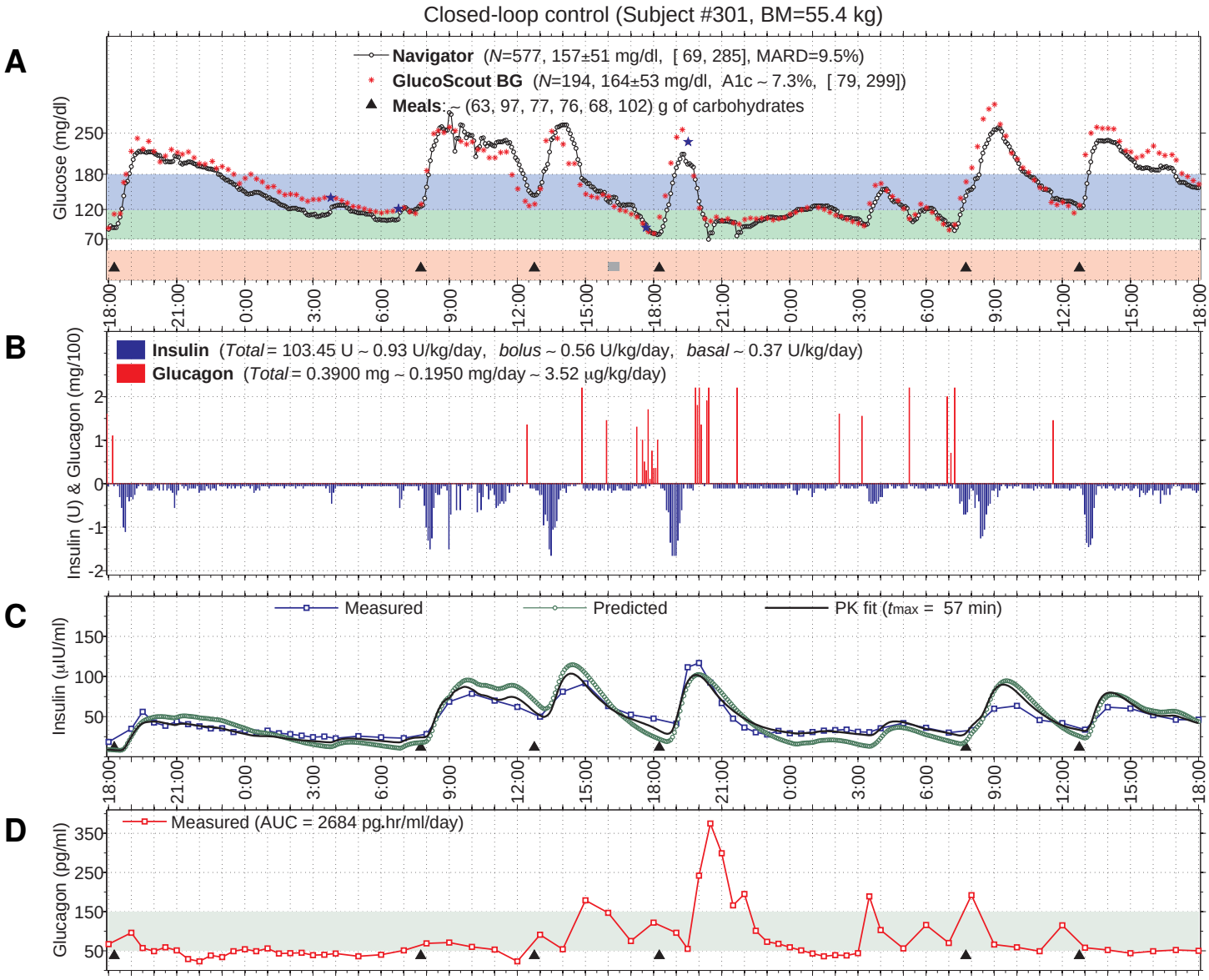

Fig. S21. 48-hour closed-loop experiment in pediatric female #301 with NMB. Respective means in CGMG and PG were 167 mg/dl and 168 mg/dl in the first 24-hour period (MARD of 10%), and 147 mg/dl and 158 mg/dl in the second 24-hour period (MARD of 9%), with respective total insulin and glucagon doses being 0.89 U/kg and 0.12 mg in the first 24-hour period, and 0.98 U/kg and 0.27 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment.

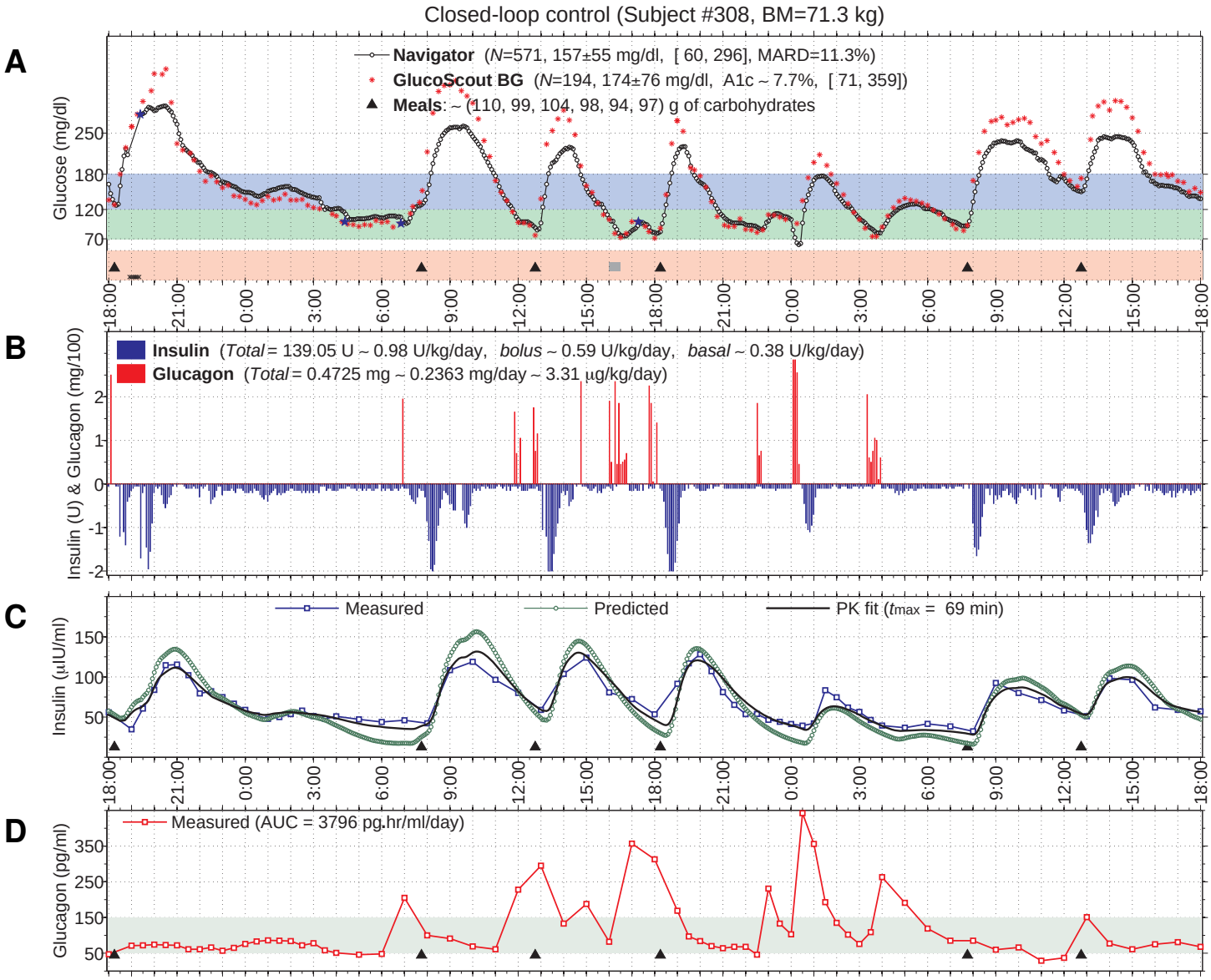

Fig. S22. 48-hour closed-loop experiment in pediatric male #308 with NMB. Respective means in CGMG and PG were 162 mg/dl and 175 mg/dl in the first 24-hour period (MARD of 12%), and 153 mg/dl and 172 mg/dl in the second 24-hour period (MARD of 11%), with respective total insulin and glucagon doses being 1.04 U/kg and 0.27 mg in the first 24-hour period, and 0.92 U/kg and 0.20 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. The Navigator was offline from 18:50 to 19:15 on Day 1. It was calibrated at 19:23. Subsequent CGMG values were lower than PG values until approximately 21:00 on Day 1.

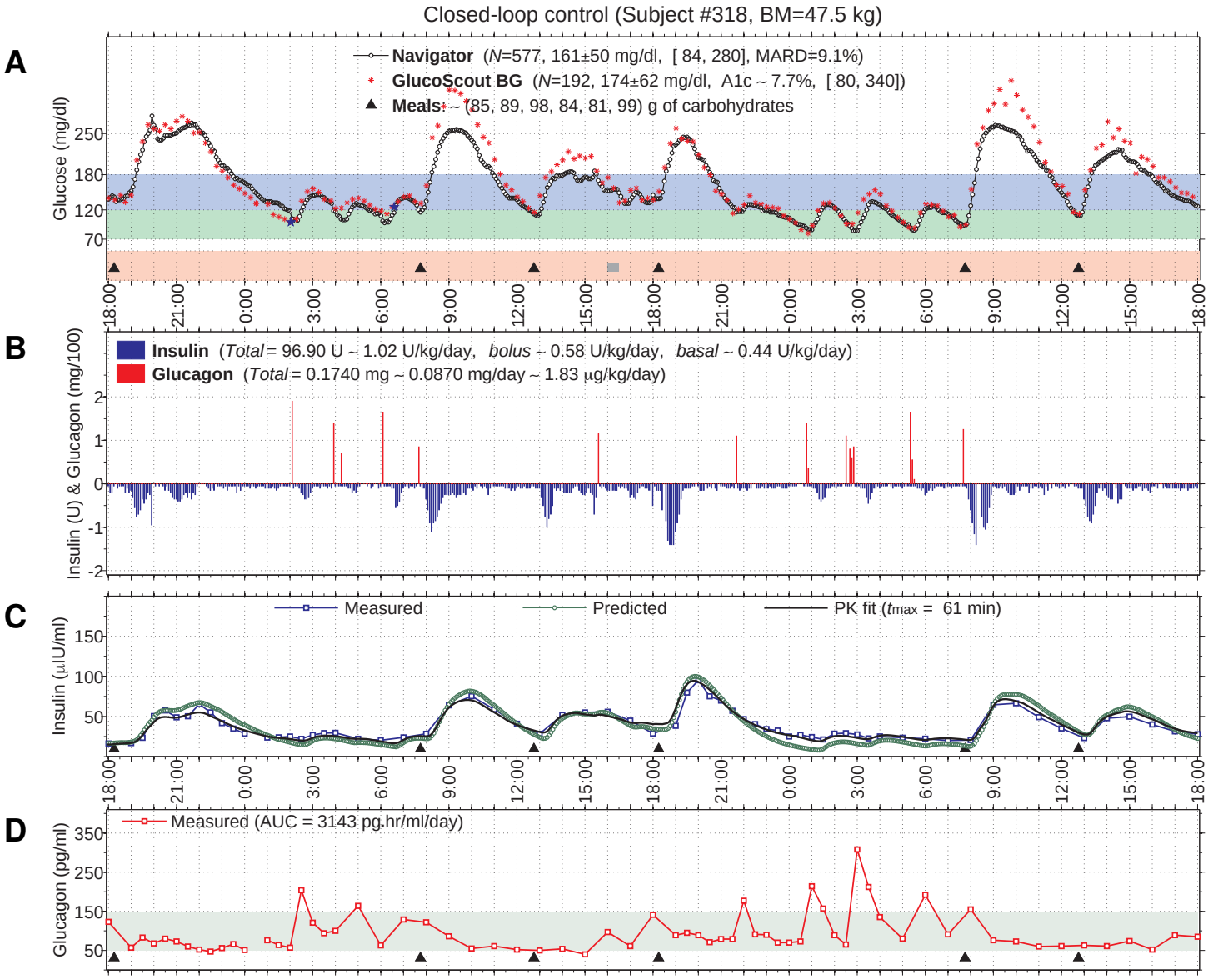

Fig. S23. 48-hour closed-loop experiment in pediatric female #318 with NMB. Respective means in CGMG and PG were 168 mg/dl and 179 mg/dl in the first 24-hour period (MARD of 10%), and 155 mg/dl and 169 mg/dl in the second 24-hour period (MARD of 9%), with respective total insulin and glucagon doses being 1.02 U/kg and 0.08 mg in the first 24-hour period, and 1.03 U/kg and 0.10 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment. The subject did have moderate ketones at 20:45 on Day 1, which cleared by 21:45 with highest PG at 279 mg/dl. The subject did experience nausea with subsequent emesis at 12:50 on Day 3. The last glucagon received was at approximately 07:30 am. CGMG and PG values remained $>$ 100 mg/dl.

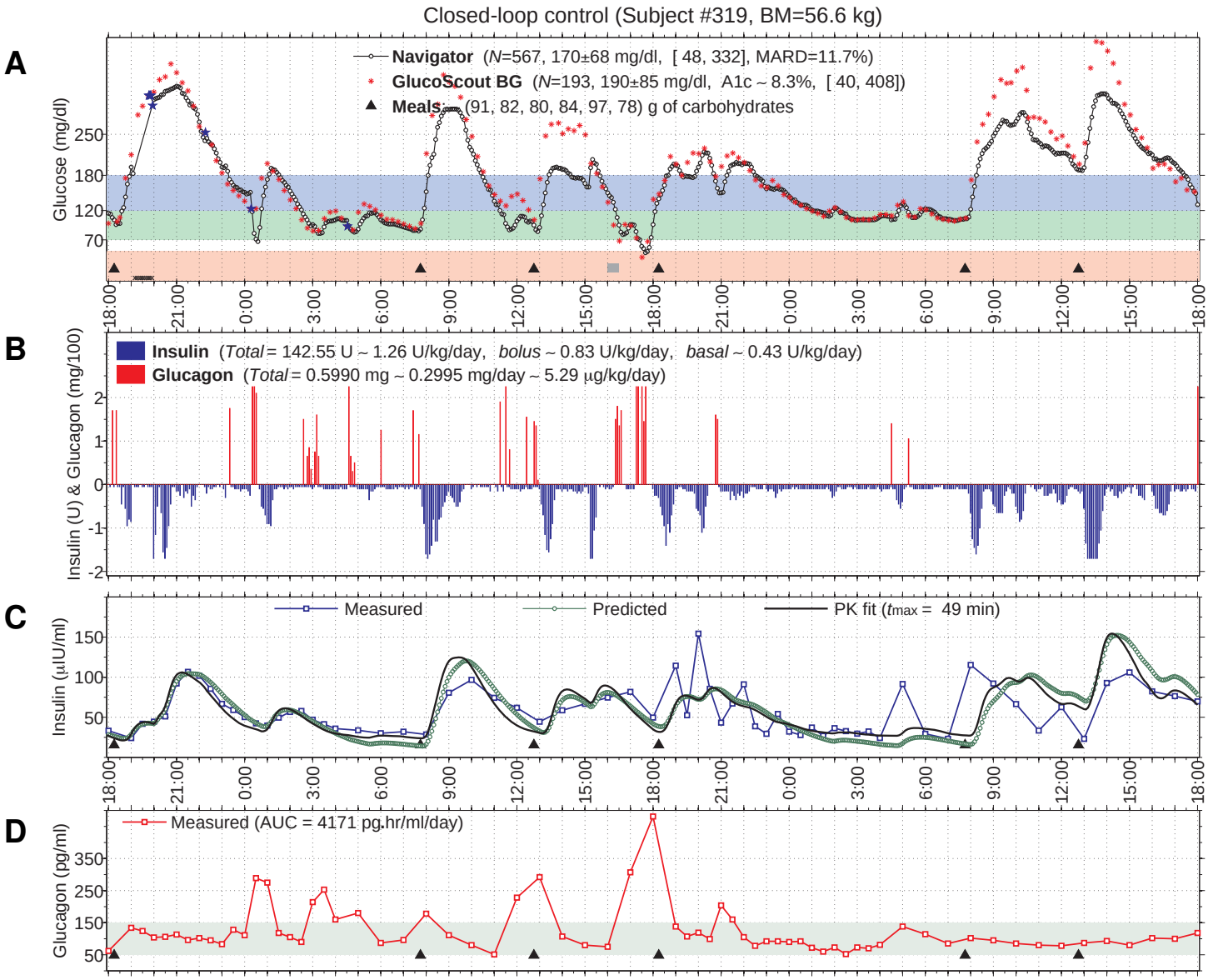

Fig. S24. 48-hour closed-loop experiment in pediatric female #319 with NMB. Respective means in CGMG and PG were 160 mg/dl and 181 mg/dl in the first 24-hour period (MARD of 15%), and 180 mg/dl and 198 mg/dl in the second 24-hour period (MARD of 9%), with respective total insulin and glucagon doses being 1.16 U/kg and 0.52 mg in the first 24-hour period, and 1.36 U/kg and 0.08 mg in the second 24-hour period. There was a single hypoglycemic episodes (venous PG $<$ 60 mg/dl), which occurred post exercise at 17:30, just before the second dinner (indicated by a small black rectangle along the timeline of Panel A and annotated with the carbohydrate content). There were no hypoglycemic episodes in the second 24-hour period in this experiment. The Navigator was offline from 19:23 to 20:00 on Day 1. A forced calibration was performed as per protocol using a PG value of 299 mg/dl (actual PG 322 mg/dl). CGMG values subsequently underestimated PG values for the next 1.5 hours until PG values declined. There was a suspected insulin pod failure which was changed at 12:15. Following PG values declined. There was a suspected insulin pod failure on Day 3 at 12:15. Glucose levels subsequently declined after the insulin pod was replaced.

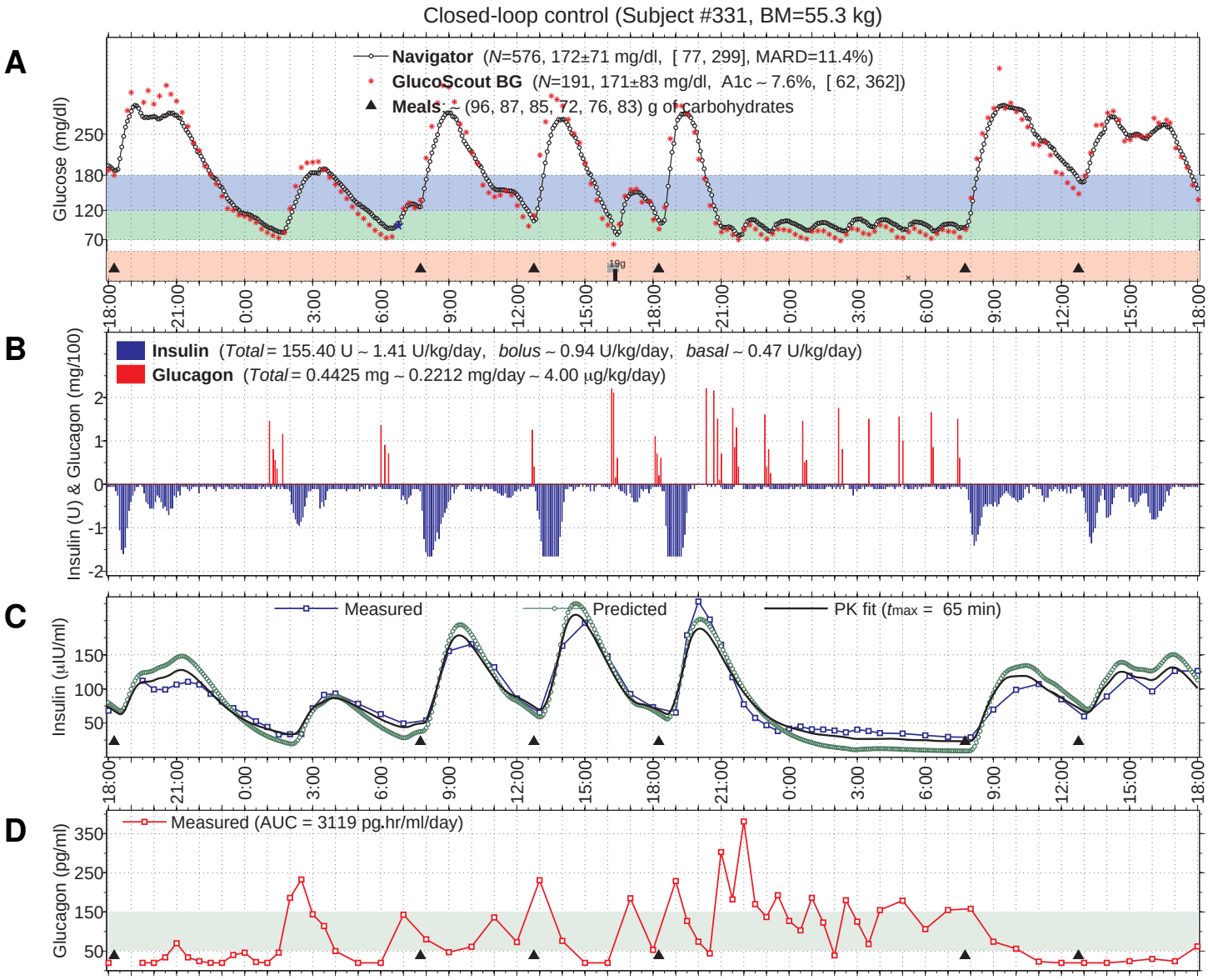

Fig. S25. 48-hour closed-loop experiment in pediatric female #331 with NMB. Respective means in CGMG and PG were 177 mg/dl and 180 mg/dl in the first 24-hour period (MARD of 11%), and 167 mg/dl and 161 mg/dl in the second 24-hour period (MARD of 12%), with respective total insulin and glucagon doses being 1.53 U/kg and 0.14 mg in the first 24-hour period, and 1.28 U/kg and 0.30 mg in the second 24-hour period. There were no hypoglycemic episodes (venous PG $<$ 60 mg/dl) in both 24-hour periods in this experiment, although there was a carbohydrate intervention (indicated by a small black rectangle along the timeline of Panel A and annotated with the carbohydrate content) administered at 16:15 in the first 24-hour period in response to the subject complaining of feeling “low”. Subjects PG value at that time was 62 mg/dl. There were no hypoglycemic episodes in the second 24-hour period in this experiment. The subject complained of a headache at 22:00 on Day 1, which resolved with ibuprofen. No glucagon had been given up to that point of the experiment. The glucagon pod failed on Day 2 and was subsequently changed (PG remained $>$ 73 mg/dl). The subject complained of nausea at 23:15 on Day 2 with a PG level at that time of 80 mg/dl. Glucagon was actively being dosed at that time.

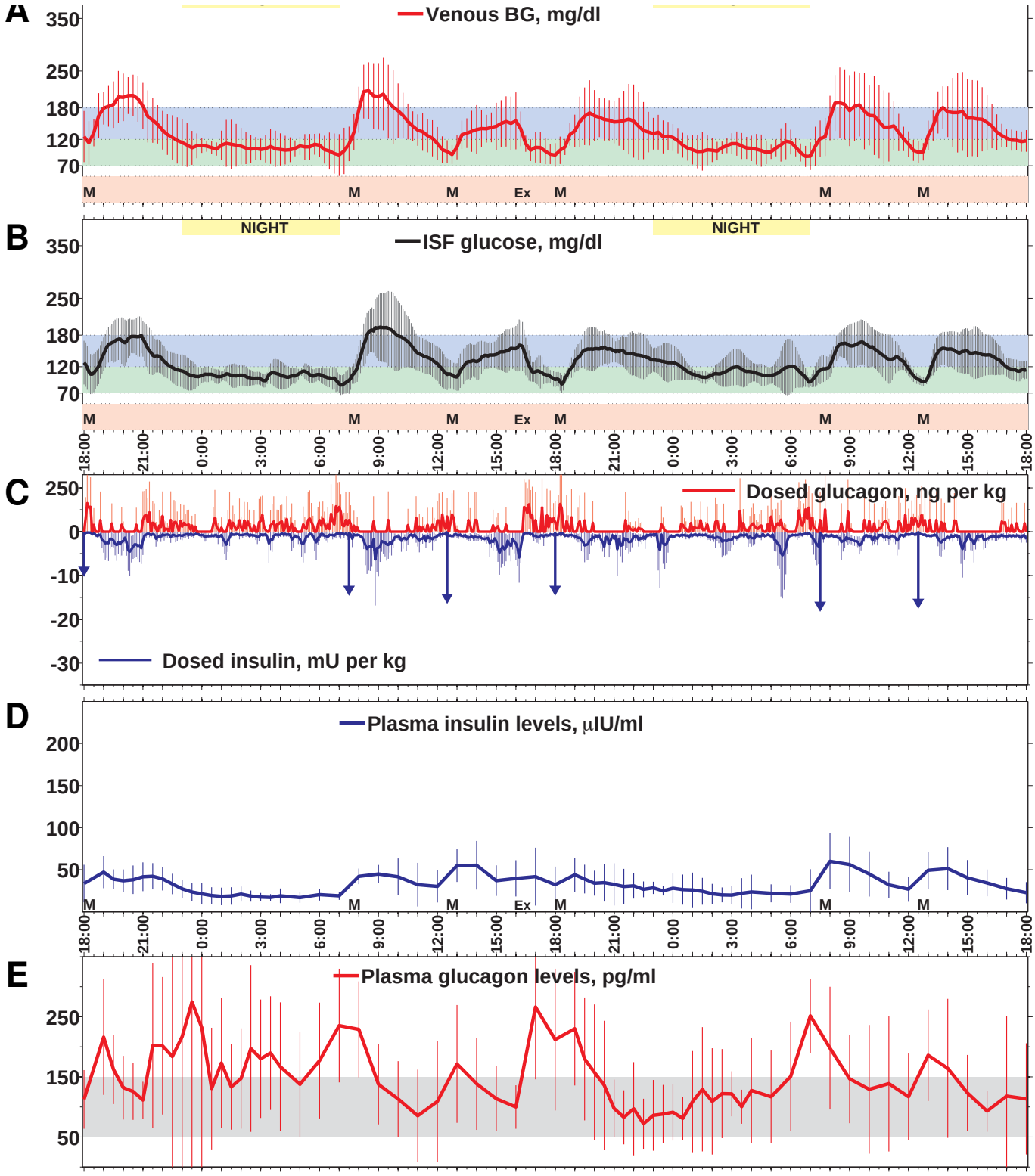

Fig. S26. Mean results of six 48-hour closed-loop experiments in adults with AMB. (**A**) shows 48-hour mean venous PG levels of the six experiments superimposed with standard deviation intervals indicated around individual 15-minute samples (N=193). The peak mean PG was 213 mg/dl, at 8:30 AM after the first breakfast, and the nadir was 88 mg/dl, at 6:45 AM before second breakfast. The overall mean of the mean PG trace was 133±32 mg/dl (N=193), and 106±9 mg/dl during the two nights (N=66). The six meals are indicated by black triangles. (**B**) shows mean 5-minute CGMG levels (N=577) that provided the closed-loop input signal, with individual standard deviation intervals. The peak mean CGMG was 195 mg/dl, at 9:05 AM after the first breakfast, and the nadir was 85 mg/dl, at 7:05 AM before the first breakfast. The overall mean of the mean CGMG trace was 128±26 mg/dl (N=577), and 108±9 mg/dl during the two nights (N=194). (**C**) shows the means of all subcutaneous insulin–glucagon doses (including meal-priming insulin doses, indicated by downward arrows) that were administered by the closed-loop system in response to the CGMG signal in (B). As a daily average, the system administered 0.64±0.27 U/kg of insulin and 6.75±9.67 μg/kg of glucagon. (**D** and **E**) show mean plasma insulin and glucagon levels, respectively, superimposed with individual standard deviation intervals (N=67). The overall mean was 32±11 μIU/ml for insulin and 149±50 pg/ml for glucagon. The t_{max} for insulin absorption ranged between 35–73 min in these six subjects, and was 61±14 min on average (t_{max} was non-convergent in most cases for glucagon), which is very close to the inherent global assumption of 65 min in the closed-loop algorithm. Although the overall mean plasma glucagon is within presumed normal range (50–150 pg/ml), it did rise on several occasions above range, owing to periods of increased glucagon dosing that can be seen in (C).

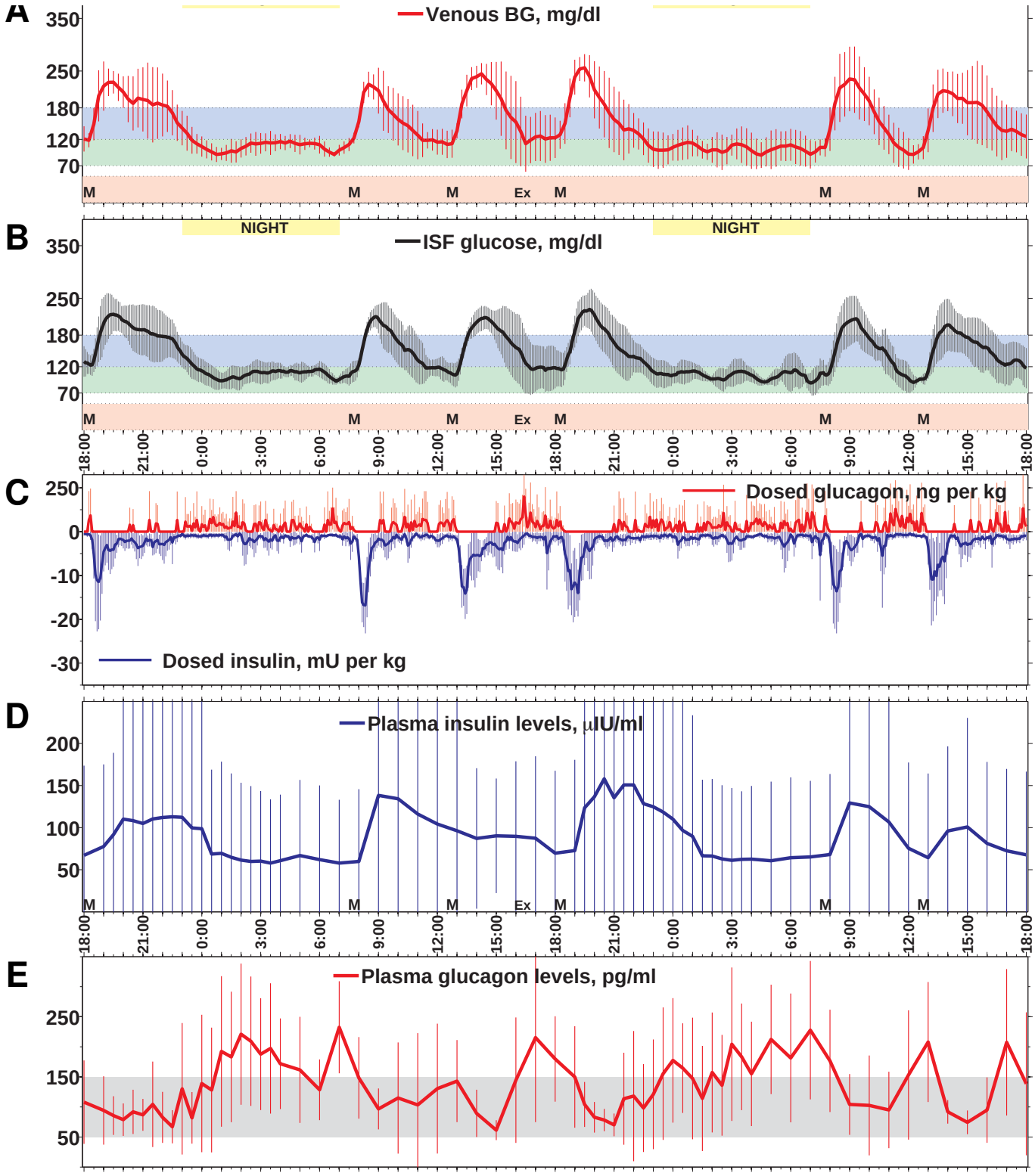

Fig. S27. Mean results of six 48-hour closed-loop experiments in adults with NMB. (**A**) shows 48-hour mean venous PG levels of the six experiments superimposed with standard deviation intervals indicated around individual 15-minute samples (N=193). The peak mean PG was 256 mg/dl, at 7:30 PM after the second dinner, and the nadir was 90 mg/dl, at 4:30 AM before second breakfast. The overall mean of the mean PG trace was 146±46 mg/dl (N=193), and 106±9 mg/dl during the two nights (N=66). The six meals are indicated by black triangles. (**B**) shows mean 5-minute CGMG levels (N=577) that provided the closed-loop input signal, with individual standard deviation intervals. The peak mean CGMG was 229 mg/dl, at 7:45 PM after the second dinner, and the nadir was 89 mg/dl, at 7:00 AM before the second breakfast. The overall mean of the mean CGMG trace was 140±39 mg/dl (N=577), and 106±9 mg/dl during the two nights (N=194). (**C**) shows the means of all subcutaneous insulin–glucagon doses that were administered by the closed-loop system in response to the CGMG signal in (B). As a daily average, the system administered 0.70±0.77 U/kg of insulin and 6.60±8.42 μg/kg of glucagon. (**D** and **E**) show mean plasma insulin and glucagon levels, respectively, superimposed with individual standard deviation intervals (N=67). The overall mean was 91±28 μIU/ml for insulin and 137±47 pg/ml for glucagon. The t_{max} for insulin absorption ranged between 36–134 min in these six subjects, and was 78±38 min on average (t_{max} was non-convergent in most cases for glucagon), which is not far from the inherent global assumption of 65 min in the closed-loop algorithm. Although the overall mean plasma glucagon is within presumed normal range (50–150 pg/ml), it did rise on several occasions above range, owing to periods of increased glucagon dosing that can be seen in (C).

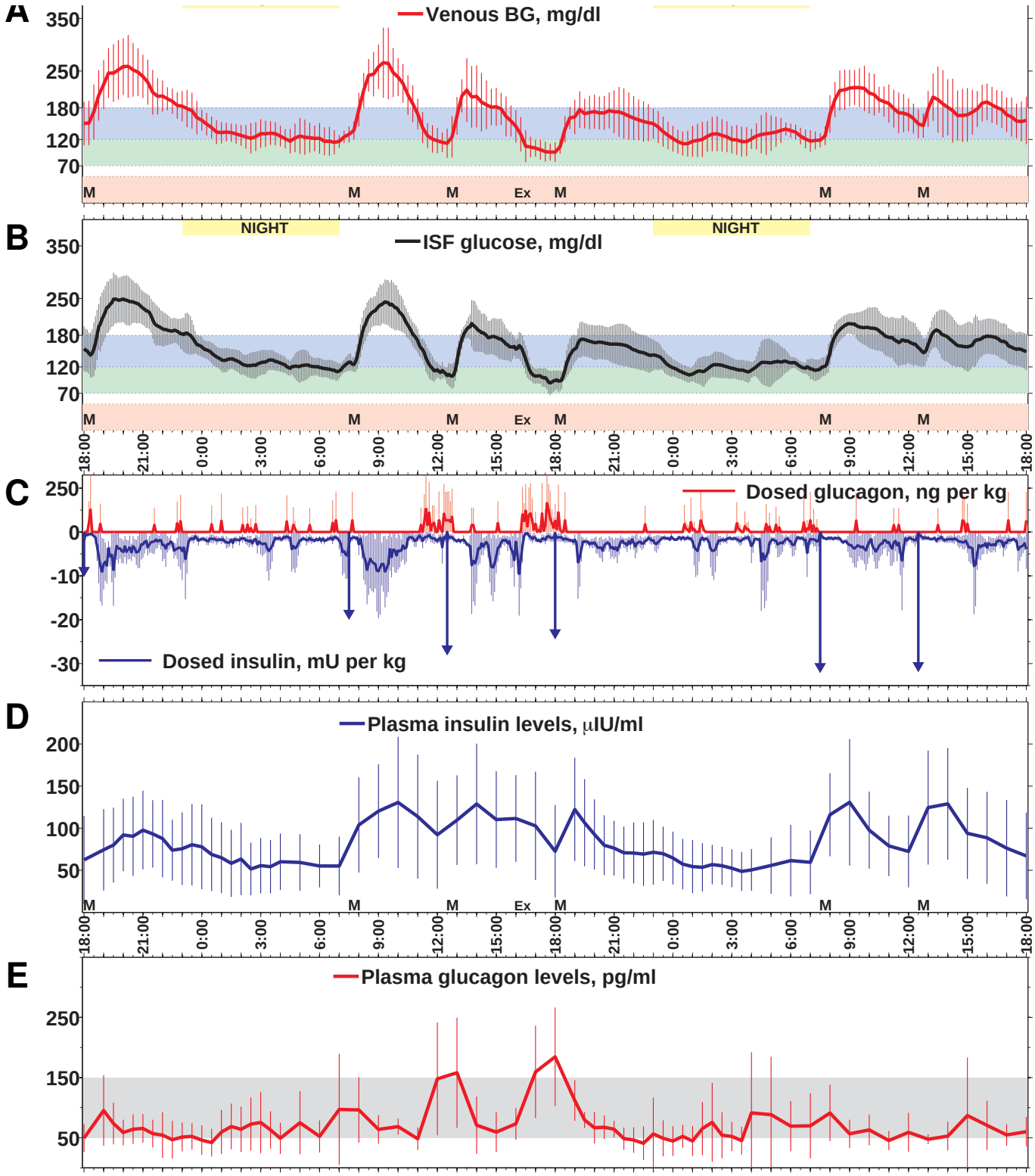

Fig. S28. Mean results of six 48-hour closed-loop experiments in pediatrics with AMB. (**A**) shows 48-hour mean venous PG of the six experiments superimposed with standard deviation intervals indicated around individual 15-minute samples (N=193). The peak mean PG was 266 mg/dl, at 9:15 AM after the first breakfast, and the nadir was 96 mg/dl, at 6:00 PM post exercise before the second dinner. The overall mean of the mean PG trace was 162$\pm$41 mg/dl (N=193), and 130$\pm$15 mg/dl during the two nights (N=66). The six meals are indicated by black triangles. (**B**) shows mean 5-minute CGMG levels (N=577) that provided the closed-loop input signal, with individual standard deviation intervals. The peak mean CGMG was 249 mg/dl, at 7:35 PM after the first dinner, and the nadir was 90 mg/dl, at 5:45 PM post exercise before the second dinner. The overall mean of the mean CGMG trace was 156$\pm$38 mg/dl (N=577), and 127$\pm$16 mg/dl during the two nights (N=194). (**C**) shows the means of all subcutaneous insulin–glucagon doses (including meal-priming insulin doses, indicated by downward arrows) that were administered by the closed-loop system in response to the CGMG signal in (B). As a daily average, the system administered 1.16$\pm$0.48 U/kg of insulin and 2.48$\pm$6.68 μg/kg of glucagon. (**D** and **E**) show mean plasma insulin and glucagon levels, respectively, superimposed with individual standard deviation intervals (N=67). The overall mean was 81$\pm$24 μIU/ml for insulin and 69$\pm$28 pg/ml for glucagon. The t_{max} for insulin absorption ranged between 42–118 min in these six subjects, and was 68$\pm$27 min on average (t_{max} was non-convergent in most cases for glucagon), which is very close to the inherent global assumption of 65 min in the closed-loop algorithm. On average, the plasma glucagon levels intermittently rose above presumed normal range (50–150 pg/ml) to a global peak of 184 pg/ml at 6:00 PM post exercise, owing to increased glucagon dosing, as can be seen in (C).

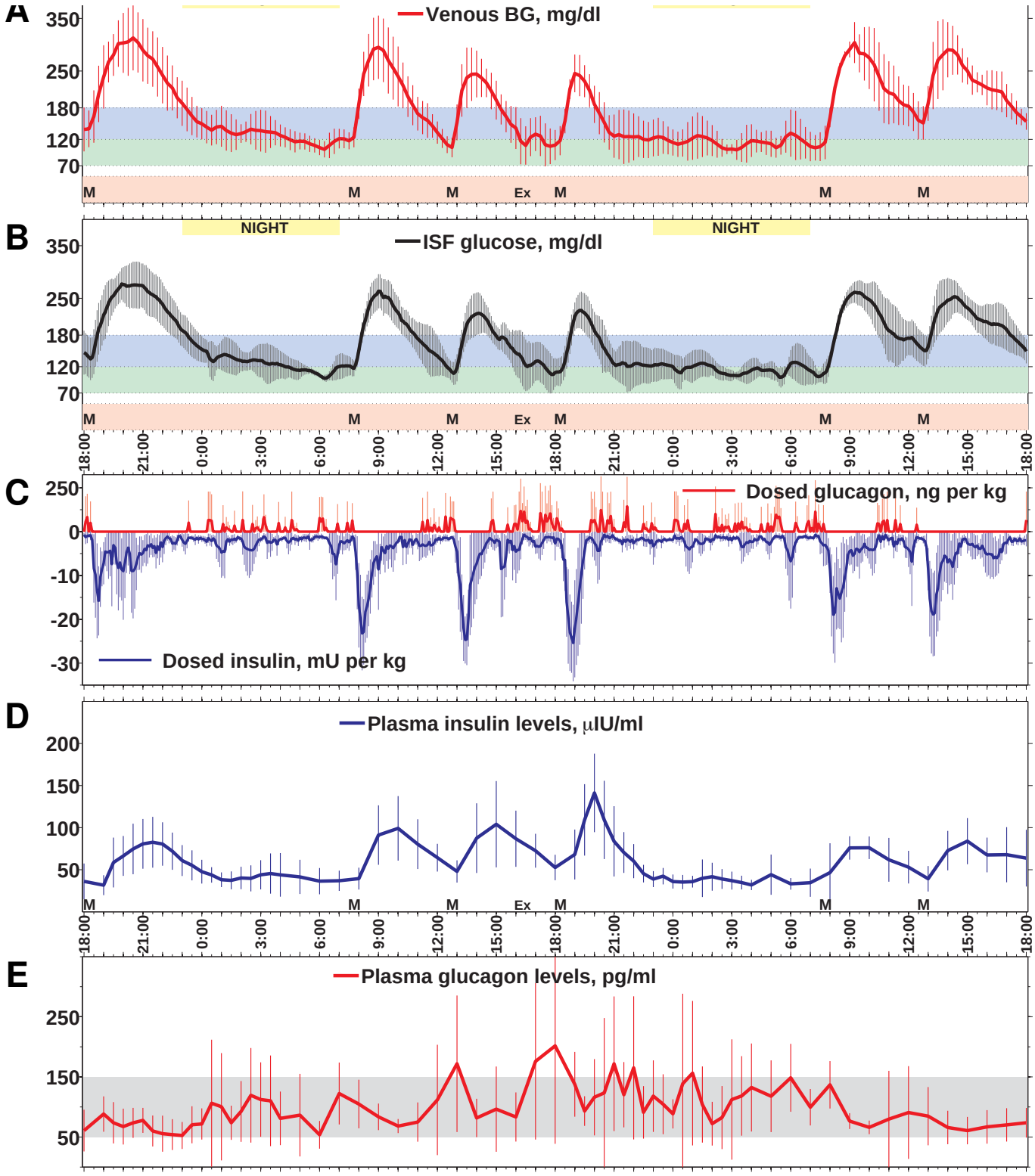

Fig. S29. Mean results of six 48-hour closed-loop experiments in pediatrics with NMB. (**A**) shows 48-hour mean venous PG of the six experiments superimposed with standard deviation intervals indicated around individual 15-minute samples (N=193). The peak mean PG was 313 mg/dl, at 8:30 PM after the first dinner, and the nadir was 100 mg/dl, at 3:15 AM before second breakfast. The overall mean of the mean PG trace was 175$\pm$63 mg/dl (N=193), and 124$\pm$17 mg/dl during the two nights (N=66). The six meals are indicated by black triangles. (**B**) shows mean 5-minute CGMG levels (N=577) that provided the closed-loop input signal, with individual standard deviation intervals. The peak mean CGMG was 277 mg/dl, at 7:55 PM after the first dinner, and the nadir was 98 mg/dl, at 6:20 AM before the first breakfast. The overall mean of the mean CGMG trace was 165$\pm$52 mg/dl (N=577), and 123$\pm$17 mg/dl during the two nights (N=194). (**C**) shows the means of all subcutaneous insulin–glucagon doses that were administered by the closed-loop system in response to the CGMG signal in (B). As a daily average, the system administered 1.16$\pm$1.24 U/kg of insulin and 3.37$\pm$7.12 μg/kg of glucagon. (**D** and **E**) show mean plasma insulin and glucagon levels, respectively, superimposed with individual standard deviation intervals (N=67). The overall mean was 59$\pm$23 μIU/ml for insulin and 99$\pm$34 pg/ml for glucagon. The t_{max} for insulin absorption ranged between 49–69 min in these six subjects, and was 60$\pm$7 min on average (t_{max} was non-convergent in most cases for glucagon), which is very close to the inherent global assumption of 65 min in the closed-loop algorithm. On average, the plasma glucagon levels intermittently rose above presumed normal range (50–150 pg/ml) to a global peak of 202 pg/ml at 6:00 PM post exercise, owing to increased glucagon dosing, as can be seen in (C).

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