Dual-Hormone Closed-Loop System Using a Liquid Stable Glucagon Formulation Versus Insulin-Only Closed-Loop System Compared With a Predictive Low Glucose Suspend System: An Open-Label, Outpatient, Single-Center, Crossover, Randomized Controlled Trial

Leah M. Wilson,[1] Peter G. Jacobs,[2] Katrina L. Ramsey,[3] Navid Resalat,[2] Ravi Reddy,[2] Deborah Branigan,[1] Joseph Leitschuh,[2] Virginia Gabo,[1] Florian Guillot,[1] Brian Senf,[1] Joseph El Youssef,[1,2] Isabelle Isa Kristin Steineck,[4] Nichole S. Tyler,[2] and Jessica R. Castle[1]

Diabetes Care 2020;43:2721–2729 | https://doi.org/10.2337/dc19-2267

OBJECTIVE

To assess the efficacy and feasibility of a dual-hormone (DH) closed-loop system with insulin and a novel liquid stable glucagon formulation compared with an insulin-only closed-loop system and a predictive low glucose suspend (PLGS) system.

RESEARCH DESIGN AND METHODS

In a 76-h, randomized, crossover, outpatient study, 23 participants with type 1 diabetes used three modes of the Oregon Artificial Pancreas system: *1*) dual-hormone (DH) closed-loop control, *2*) insulin-only single-hormone (SH) closed-loop control, and *3*) PLGS system. The primary end point was percentage time in hypoglycemia (<70 mg/dL) from the start of in-clinic aerobic exercise (45 min at 60% VO_{2max}) to 4 h after.

RESULTS

DH reduced hypoglycemia compared with SH during and after exercise (DH 0.0% [interquartile range 0.0–4.2], SH 8.3% [0.0–12.5], $P = 0.025$). There was an increased time in hyperglycemia (>180 mg/dL) during and after exercise for DH versus SH (20.8% DH vs. 6.3% SH, $P = 0.038$). Mean glucose during the entire study duration was DH, 159.2; SH, 151.6; and PLGS, 163.6 mg/dL. Across the entire study duration, DH resulted in 7.5% more time in target range (70–180 mg/dL) compared with the PLGS system (71.0% vs. 63.4%, $P = 0.044$). For the entire study duration, DH had 28.2% time in hyperglycemia vs. 25.1% for SH ($P = 0.044$) and 34.7% for PLGS ($P = 0.140$). Four participants experienced nausea related to glucagon, leading three to withdraw from the study.

CONCLUSIONS

The glucagon formulation demonstrated feasibility in a closed-loop system. The DH system reduced hypoglycemia during and after exercise, with some increase in hyperglycemia.

[1]Harold Schnitzer Diabetes Health Center, Division of Endocrinology, Oregon Health & Science University, Portland, OR
[2]Artificial Intelligence for Medical Systems (AIMS) Lab, Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR
[3]Oregon Clinical and Translational Research Institute Biostatistics and Design Program, Oregon Health & Science University & Portland State University School of Public Health, Portland, OR
[4]Steno Diabetes Center Copenhagen, Gentofte, Denmark

Corresponding author: Leah M. Wilson, wilsolea@ohsu.edu

Received 11 November 2019 and accepted 16 August 2020

Clinical trial reg. no. NCT03424044, clinicaltrials.gov

This article contains supplementary material online at https://doi.org/10.2337/figshare.12815861.

L.M.W. and P.G.J. share co-first authorship.

Closed-loop insulin delivery systems have shown promise in improving glucose control for people with type 1 diabetes. These systems deliver insulin automatically based on glucose values from a continuous glucose monitor (CGM). Despite this adaptive delivery of insulin, hypoglycemia can still occur in part due to both the slow onset and offset of short-acting insulin formulations and the dysregulation and loss of glucagon secretion that occurs early in the course of type 1 diabetes. Several research groups are working on closed-loop systems that automatically deliver glucagon in addition to insulin to further reduce hypoglycemia (1–18). These dual-hormone (DH; insulin and glucagon) systems are likely to be beneficial in circumstances with high risk for hypoglycemia, such as during exercise. Many people with type 1 diabetes experience hypoglycemia related to exercise. This may lead to avoidance of exercise. The physiologic changes with exercise, including insulin-independent glucose utilization by muscle and enhanced insulin sensitivity, make avoidance of hypoglycemia during exercise challenging (19).

Previous work from our group and others has shown that the addition of glucagon to a closed-loop system can reduce hypoglycemia during aerobic exercise (1,3,4). These prior studies relied on lyophilized glucagon preparations from emergency hypoglycemia rescue kits. This form of glucagon must be reconstituted every 24 h and the pump systems refilled and replaced. Real-world use of a DH system requires a glucagon product that can be inserted into a pump system and remains stable. Several liquid stable glucagon products are under development that meet these requirements, including the formulation used in this study (20).

We report here the results of a single-center, randomized, open-label trial of a single-hormone (SH) and DH closed-loop system compared with a predictive low glucose suspend (PLGS) system in adults with type 1 diabetes in an outpatient setting with structured aerobic exercise. The aim of this study was twofold; firstly, it was to evaluate the latest iteration of our SH and DH closed-loop system compared with the PLGS control system, and secondly, to evaluate the feasibility of the liquid stable glucagon formulation in this context. We hypothesized that the DH closed-loop system with automated insulin and stable liquid glucagon delivery would reduce time in hypoglycemia during and immediately after exercise.

RESEARCH DESIGN AND METHODS

Participants and Study Design

This study was a single-center, open-label, randomized controlled trial with adults ages 21–50 with type 1 diabetes recruited from Oregon Health & Science University (OHSU) and surrounding area. From 30 April 2018 to 16 May 2019, 23 adults with type 1 diabetes were enrolled. All participants provided written informed consent before participating in the study. This study was conducted under a U.S. Food and Drug Administration-approved investigation device exemption and with OHSU Institutional Review Board (Portland, OR) approval. This trial is registered with ClinicalTrials.gov, number NCT03424044.

Briefly, inclusion criteria included use of an insulin pump for >3 months, A1C ≤10%, using <139 units of insulin per day, and willing and able to perform 45 min of exercise. Exclusion criteria included pregnancy or intention of becoming pregnant, cardiovascular, liver, or kidney disease, anemia, uncontrolled hypertension, history of diabetic ketoacidosis within 6 months, or severe hypoglycemia within 12 months. Full inclusion and exclusion criteria are available in Supplementary Fig. 1. Of the 23 participants who passed all screening, 18 participants completed all three study arms, and 1 additional participant completed all three arms with the exception of 24 h of one arm. All participants who completed the time period necessary for assessment of the primary end point (day 1 exercise plus 4 h) were included in the data analysis (DH $n = 19$, PLGS $n = 20$, SH $n = 21$). See Table 1 for baseline characteristics. See Supplementary Fig. 2 for enrollment diagram.

Table 1—Baseline characteristics of study participants

Characteristic	Mean (SD)
Age (years)	32.4 (7.0)
Weight (kg)	80.1 (14.7)
Sex, *n*	
Male	13
Female	10
A1C (%)	7.1 (0.9)
Duration of diabetes (years)	16.7 (8.1)

Randomization and Masking

Participants were randomized to study arm order by a blocked scheme in two orthogonal Latin squares to balance possible crossover effects. Randomization was done electronically by a third party not involved in the study procedures. Owing to the nature of the interventions, the participants and study physicians were not masked to group assignment.

Procedures

Participants underwent three ~76-h study arms. See Supplementary Fig. 3 for a diagram of study structure. In randomized order, participants used one of the following systems: *1*) DH insulin and glucagon closed-loop, *2*) insulin-only SH closed-loop, and *3*) insulin-only PLGS system. Participants in all three study arms wore a G6 sensor (Dexcom, San Diego, CA). There was a 1-week run-in period before the first study arm where the participants used CGM through the study software for training purposes. Approximately 24 h before the second and third study arms, participants inserted the device at home before the start of the study. CGM data were transmitted to the study phone, and these data were visible by the patient in all study arms. On day 1 of each study arm, participants remained in the clinical research center for ~11 h. The pump(s) were placed at ~8:00 A.M. on day 1 of each study, ~30 min before the breakfast meal. A Zephyr BioPatch (Medtronic, Boulder, CO) was placed at the start of the study. Participants were asked to wear the Zephyr for the duration of the study apart from overnight charging. Participants self-selected breakfast, lunch, and dinner and consumed these same meals on day 1 of each study arm. Participants ate lunch at ~12:00 P.M. Then at ~2:00 P.M., participants performed aerobic exercise on a treadmill for 45 min at 60% of their VO_{2max}. The heart rate required to reach 60% of their VO_{2max} was determined by VO_{2max} testing completed just after study enrollment. CGM alerts were set at 70 mg/dL and 300 mg/dL. A capillary blood glucose (CBG) was measured for CGM alerts <70 mg/dL. Participants were treated with 15 g of carbohydrate for CBG values <70 mg/dL. During the first 11 h on day 1, no carbohydrates were permitted aside from those included in the meals. After this initial period, there were no restrictions on carbohydrate intake. No adjustments to the lunch insulin bolus or PLGS basal rates were allowed before in-clinic exercise in any of the study arms.

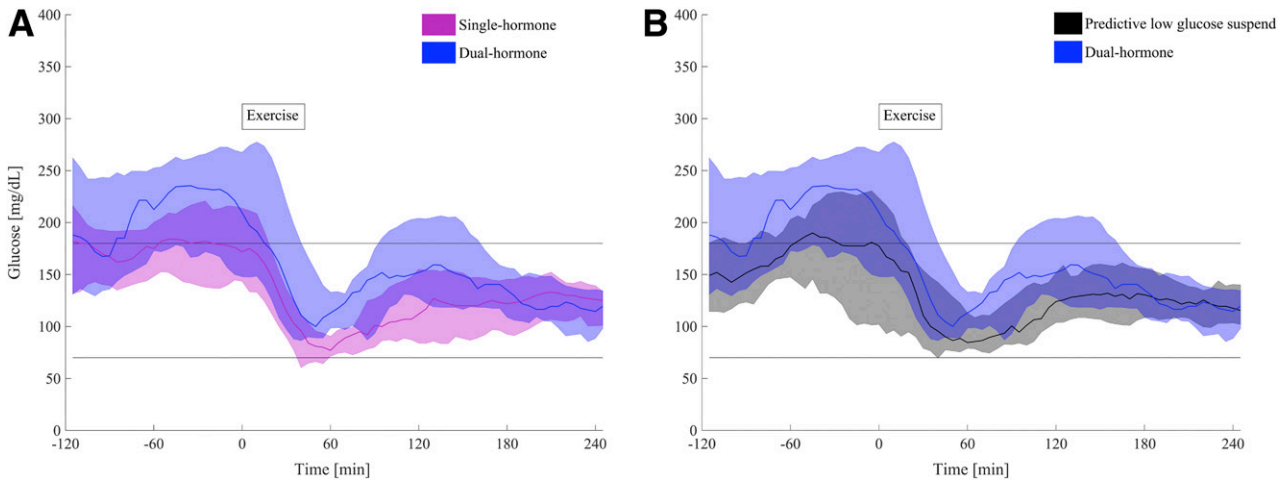

Figure 1—Glucose from 2 h before the start of in-clinic exercise to 4 h after exercise. *A*: SH (magenta) vs. DH (blue). *B*: PLGS (black) vs. DH (blue). "Exercise" box indicates the 45-min exercise session. The shaded areas show the 25% and 75% interquartile ranges. The upper black line indicates 180 mg/dL, and the lower black line indicates 70 mg/dL.

System Description

The closed-loop system is a modified fading memory proportional-derivative algorithm, as previously described (21). The PLGS system was also previously described (1). The PLGS algorithm is an in-house designed algorithm modeled after the Medtronic 640G system (22). In brief, the PLGS system suspends insulin when glucose is 70–140 mg/dL and predicted to drop to <90 mg/dL within 30 min. Insulin delivery resumes when glucose is 70–140 mg/dL and predicted to rise >120 mg/dL within 30 min. Prediction of glucose is based on linear regression of the previous 10 min of CGM data. The control algorithm was run on a LG Nexus 5 smartphone (Google, Mountain View, CA), which communicated via Bluetooth Low Energy (BLE) relay Personal Diabetes Manager then to OmniPod pod(s) (Insulet, Boston, MA) to adjust delivery rates every 5 min based on Dexcom G6 readings. The insulin pods were filled with insulin aspart (Novo Nordisk, Plainsboro, NJ), and for the DH system, the pods were filled with XeriSol glucagon (Xeris Pharmaceuticals, Chicago, IL). This liquid stable formulation of glucagon is produced by solid-phase synthesis and has an identical amino acid sequence to the human peptide, which is dissolved in an aprotic polar solution. It is room temperature shelf-stable for up to 2 years. Because the ready-to-use glucagon does not fibrillate, no pod exchanges are required for this liquid stable glucagon formulation. Participants were remotely monitored in all study arms. Alarms were sent to study staff if the CGM reading was <40 mg/dL or >400 mg/dL or if the participant did not respond to system alarms.

Exercise Prediction and Modified Dosing

The SH and DH closed-loop algorithms used a previously described automated exercise detection algorithm with inputs from the Zephyr BioPatch (heart rate and accelerometer) (1,23). Exercise was detected if the MET >4 for ≥5 min, The system then prompted the subject to confirm whether they were exercising. If exercise was confirmed, insulin was suspended for 30 min and then reduced by 50% of the typical rate for 60 min. These adjustments are based on previous in silico simulations (23). For the DH system, these same insulin adjustments were made, and additionally, the target glucose for glucagon delivery was increased from 95 to 120 mg/dL and the maximum glucagon dose was increased by a factor of 2. In a prespecified adaptation, if hypoglycemia (<70 mg/dL) occurred with exercise on day 1, then the glucose target for glucagon delivery was increased from 120 to 130 mg/dL. No proactive carbohydrate intake before in-clinic exercise was allowed in any of the study arms.

In addition to the changes described above, a hypoglycemia prediction feature was used for exercise adaptation for the DH system (24). This used a random forest model that was trained, tested, and validated from prior closed-loop study data sets to predict the occurrence of hypoglycemia (<70 mg/dL) with exercise. If hypoglycemia was predicted with exercise, then a maximum possible dose of 73 μg of glucagon was dosed proactively with all safety requirements for glucagon dosing in place to prevent overdosing. The glucagon amount of 73 μg was selected by running simulations on the OHSU virtual patient population (25) to minimize time in hypoglycemia and maximize time in range. The hypoglycemia prediction feature operated every 5 min during the 30 min after the start of exercise. If hypoglycemia was predicted any time during this window, then additional glucagon was dosed as described.

Meal Adaptation

For all control modes of the Oregon Artificial Pancreas Control system, meal intake was announced in grams of carbohydrates. An adaptive learning postprandial hypoglycemia prevention algorithm (ALPHA) was used to adapt postprandial insulin delivery after meals (26) in closed-loop modes. If hypoglycemia or hyperglycemia was observed after a meal, then insulin after the next meal was adjusted using an aggressiveness factor to reduce or increase the postprandial insulin delivered, respectively. ALPHA was initiated anew with each closed-loop study arm, with no adaptive changes passed between these studies.

Outcomes

The prespecified primary outcome for this study was the percentage of time in hypoglycemia (<70 mg/dL), based on CGM values, from the start of exercise to 4 h after the start of exercise for the in-clinic exercise session. Prespecified

Table 2—Comparisons of DH system, SH system, PLGS system, and current care

							PLGS-DH			SH-DH		
Entire study period	DH (n = 19)		SH (n = 21)		PLGS (n = 20)		Paired difference# or paired ratio† (95% CI)		P value	Paired difference# or paired ratio† (95% CI)		P value
% Time in range (70–180 mg/dL)**	71.0	(9.2)	72.6	(9.2)	63.4	(16.6)	−7.5	(−14.9 to −0.2)	**0.044**	1.6	(−2.1 to 5.2)	0.400
Events of extreme hypoglycemia (<54 mg/dL)	0.5	(0.8)	1.6	(2.1)	1.3	(1.5)	2.5	(1.1–6.1)†	**0.037**	3.2	(1.4–7.5)†	**0.006**
% Extreme hypoglycemia (<54 mg/dL)	0.0	(0.0–0.2)	0.2	(0.0–0.8)	0.1	(0.0–0.8)			**0.042**			**0.012**
% Hypoglycemia (<70 mg/dL)	0.5	(0.0–0.9)	1.3	(0.5–2.8)	1.5	(0.3–2.9)			**0.019**			**0.005**
% Hyperglycemia (>180 mg/dL)	28.2	(9.4)	25.1	(9.0)	34.7	(16.7)			0.140			**0.044**
% Extreme hyperglycemia (>250 mg/dL)	7.2	(4.1–10.3)	4.7	(3.2–6.7)	8.2	(3.6–13.9)			0.780			**0.036**
Mean glucose (mg/dL)	159.2	(13.4)	151.6	(12.3)	163.6	(21.1)	4.6	(−5.2 to 14.3)	0.360	−7.6	(−12.4 to −2.8)	**0.002**
Exercise +4 h	DH		SH		PLGS		PLGS-DH			SH-DH		
% Time in range (70–180 mg/dL)	77.1	(52.1–95.8)	85.4	(79.2–91.7)	85.4	(75.0–94.8)			0.080			0.410
% Extreme hypoglycemia (<54 mg/dL)	0.0	(0.0–0.0)	0.0	(0.0–2.1)	0.0	(0.0–1.0)			0.200			0.070
% Hypoglycemia (<70 mg/dL)*	0.0	(0.0–4.2)	8.3	(0.0–12.5)	4.2	(0.0–9.4)			0.080			**0.025**
% Hyperglycemia (>180 mg/dL)	20.8	(0.0–47.9)	6.3	(0.0–12.5)	4.2	(0.0–21.9)			**0.015**			**0.038**
% Extreme hyperglycemia (>250 mg/dL)	0.0	(0.0–8.3)	0.0	(0.0–0.0)	0.0	(0.0–0.0)			**0.030**			0.220
Mean glucose (mg/dL)	146.3	(34.5)	124.4	(36.6)	123.2	(29.2)	−24.1	(−38.7 to −9.6)	**0.001**	−22.0	(−35.9 to −8.0)	**0.002**
Overnight (12:00 A.M.–6:00 A.M.)	DH		SH		PLGS		PLGS-DH			SH-DH		
% Time in range (70–180 mg/dL)	100.0	(71.5–100.0)	94.4	(72.2–100.0)	62.5	(22.2–100.0)			**0.004**			0.690
% Extreme hypoglycemia (<54 mg/dL)	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.0	(0.0–0.0)			0.200			0.170
% Hypoglycemia (<70 mg/dL)	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.0	(0.0–0.0)			0.090			**0.030**
% Hyperglycemia (>180 mg/dL)	0.0	(0.0–27.8)	1.4	(0.0–20.8)	36.1	(0.0–76.4)			**0.004**			0.860
% Extreme hyperglycemia (>250 mg/dL)	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.0	(0.0–19.4)			0.070			0.670
Mean glucose (mg/dL)	144.9	(33.5)	139.3	(32.2)	168.9	(49.6)	25.0	(8.0–42.0)	**0.004**	−5.9	(−15.7 to 3.9)	0.240
Per day	DH		SH		PLGS		PLGS-DH			SH-DH		
Carbohydrate treatments[1]**	0.6	(0.9)	1.7	(2.0)	1.4	(1.3)	2.4	(1.1–5.5)†	**0.033**	3.0	(1.5–6.1)†	**0.003**
Insulin (units)	42.3	(16.9)	41.1	(16.4)	44.0	(15.3)	2.3	(−0.3 to 5.0)	0.090	0.1	(−1.3 to 1.5)	0.860
Glucagon (μg)	364.5	(170.9)										
Glucagon (μg/kg)	4.5	(1.9)										

Data are mean (SD) or median (25% to 75% quartile). No significant period effect was observed. Bold P values are statistically significant ($P < 0.05$). *Primary outcome. **Secondary outcome. [1]Carbohydrate treatments: 15 g of carbohydrate counted as one rescue carb treatment. #Unless specified otherwise, paired differences are means with CIs based on bootstrapped SEs. †Count data are presented as ratios. Where no paired differences are given, P values are from Wilcoxon matched-pairs signed rank tests, with lower P values indicating more evidence of difference between study arms.

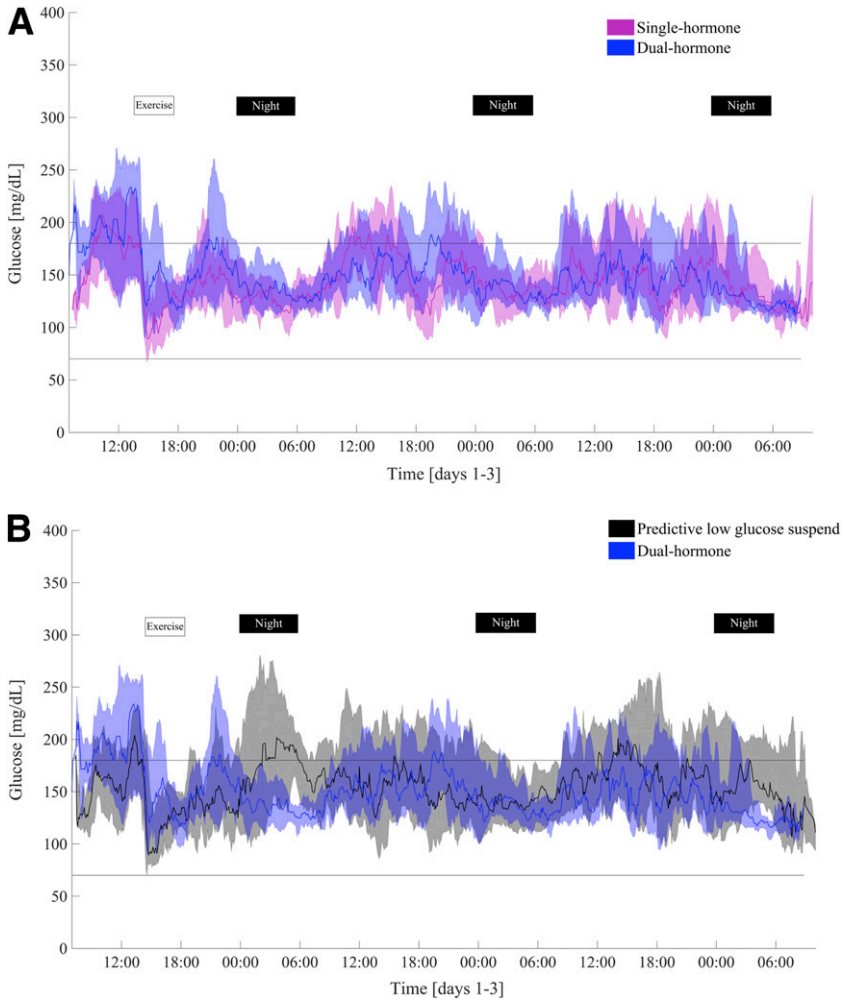

Figure 2—Glucose for full duration of study. *A*: SH (magenta) vs. DH (blue). *B*: PLGS (black) vs. DH (blue). "Exercise" box indicates exercise start until 4 h after. "Night" box indicates overnight period (12:00 A.M.–6:00 A.M.). The shaded areas show the 25% and 75% interquartile ranges. The upper black line indicates 180 mg/dL, and the lower black line indicates 70 mg/dL.

secondary outcomes for the entire study period were the percentage of time CGM data were in range (70–180 mg/dL), percentage of time with CGM data <54 mg/dL, percentage of time with CGM >180 mg/dL, and mean CGM. Prespecified secondary outcomes per day were rescue carbohydrate treatments per day (with 15 g of carbohydrate counted as one rescue carb treatment), mean amount of insulin delivered per day in units/kg, and the mean amount of glucagon delivered per day in μg/kg. Post hoc analysis included glycemic metrics between 12:00 A.M. and 6:00 A.M. and between 6:00 A.M. and 12:00 A.M. to isolate overnight glucose control.

Safety and tolerability of the stable liquid glucagon formulation were assessed by study physician evaluation for edema and/or erythema at the site of the glucagon pod on day 4 when the pod was removed. Pain and discomfort from glucagon administration was assessed with a visual analog score on discharge from inpatient unit on day 1 and study close visit on day 4. Scale was from 0 (no pain or discomfort) to 100 (worst pain or discomfort). The participants marked along a line, and a single study staff for consistency measured the distance of the mark from 0 to obtain this score. Safety end points included adverse events and severe adverse events.

Statistical Analysis

A total sample size of 19 achieved 80% power to detect a paired difference in the percentage of time in hypoglycemia of 6.1% (SD 8.9) on the absolute scale when the DH system was used during the 4 h after the start of aerobic exercise compared with PLGS. In our previous experiments, we found that participants spent an average of 3.7% time in hypoglycemia using DH (SD 4.8%) and 9.8% time in hypoglycemia (SD 9.5%) under PLGS. We expected an even greater difference between DH and SH. We did not adjust for multiplicity in these comparisons (27).

Data were analyzed using an intent-to-treat approach that included all available data from study arms in which data for the primary outcome were available. As in our prior closed-loop studies, missing CGM values due to sensor dropout or technical issues were interpolated for up to 20-min segments or longer if there were capillary blood glucose values to use as additional interpolation points during the dropout period. This approach provided the most complete data set possible; we compared results with and without the longer segments and found that conclusions were unaffected and estimates unchanged to the second or third decimal place. The 20 segments that were longer than 20 min were between 30 and 250 min long and represented 0.6% of the total observation time. Only three segments were longer than 120 min; the longest imputed segment between an available CBG or CGM value was 175 min. Graphs of these and measured glucose values, meals, exercise periods, rescue carbohydrates, and insulin boluses were generated for each subject and study arm with time on the *x*-axis and reviewed by the study team. Within-arm sample means and SD (for outcomes that were approximately normally distributed, and counts) and medians and interquartile range (for nonnormal outcomes) were calculated for measures of interest over specific time frames (i.e., entire observation period, overnight, 4 h after start of exercise, and daily).

The main measures of interest were paired differences within participants, which were first estimated as means (SD) and then tested as coefficients of indicator variables for SH and PLGS in regression models with bootstrapped variance estimates. These regression coefficients represented the mean difference from DH. The period in which each arm occurred (1, 2, or 3) was included to increase precision but did not show statistically or clinically significant effects. The bootstrap resampled participants rather than individual values to

account for the correlation between repeated observations and used 10,000 replications. Counts (numbers of extreme hypoglycemic events and carbohydrate treatments) were modeled using negative binomial regression, which yields ratios of rates instead of the linear model. For other outcomes that were not normally distributed, we performed nonparametric Wilcoxon signed rank tests for the paired differences between study arms. As sensitivity analyses, we compared the primary approach to *1*) mixed-effects regression models with robust (sandwich) variance estimates, and *2*) nonparametric tests for normally distributed outcomes. All analyses were performed using Stata/IC version 15.1 (28).

RESULTS

Between 30 April 2018 and 16 May 2019, 26 participants were screened for eligibility and 23 were enrolled and randomized to order of study arm completion. Full details of enrollment and dropout are in Supplementary Fig. 2. Demographics for randomized participants are reported in Table 1. This was a relatively young population (mean age 32.4 years) with fairly well controlled diabetes (mean A1C 7.1%). The study arms consisted of the three modes of the Oregon Artificial Pancreas system: *1*) DH insulin and glucagon, *2*) insulin-only SH, and *3*) insulin-only PLGS. Eight participants were randomized to SH first, seven to DH first, and eight to PLGS first. Eighteen participants completed all three study arms. An additional participant completed two arms and 52 h (68%) of the third arm. Data from all three arms of this participant were used for the data analysis, for a total of 19 participants providing data for all three arms. All but one study had ≥95% time in closed-loop mode; median time in closed loop was 100%. One SH study had time in closed loop of 73% related to an issue with the sensor start up. Owing to a battery issue with the initial batch of pods, early participants experienced pod failure approximately every 24 h. Once this issue was identified and a new batch of pods was acquired, there were no further premature pod failures. There were no glucagon pod failures related to occlusion.

The median time to exercise detection was 5 min (25th to 75th quartile, 3–7) because some participants exceeded the MET threshold for exercise while walking to the treadmill. The median change in glucose before exercise detection was −3.5 mg/dL (−12 to 2) (DH −5.0, SH −3.0, PLGS −3.5 mg/dL). There was an average of 1.4 false-positive alerts for exercise per day, whereby a false positive was defined as the MET threshold for exercise detection was exceeded but the user indicated that they were not exercising. The intention-to-treat analysis included all available data for 21 participants for the SH group, 19 for DH group, and 20 for PLGS group. The most common reason for participants stopping the study prematurely was nausea during the DH arm ($n = 3$). Two of these individuals contributed observations for the SH and PLGS arms before withdrawal that were near the middle of the sample distribution (33rd, 66th, and 50th percentiles within the arms). Those who did not complete all studies were somewhat older (33.8 vs. 32.4 years), had lower baseline AIC measures (6.7 vs. 7.2%), and longer duration of diabetes (21 vs. 15 years) on average. No studies were terminated early due to safety study stopping criteria.

Primary Outcome

The median percentage time in hypoglycemia (<70 mg/dL) during the 4 h after the start of exercise was lowest in the DH condition at 0.0% (0.0–4.2), followed by PLGS at 4.2% (0.0–9.4), and then SH at 8.3% (0.0–12.5) (see Fig. 1 and Table 2). Participants were hypoglycemic longer in the SH arm than in the DH arm ($P = 0.025$). The difference between PLGS and DH was not statistically significant ($P = 0.080$) (see Table 2). The mean glucose was significantly higher at the start of the study and at the start of in-clinic exercise in DH compared with SH and PLGS. Controlling for this difference in glucose at the start of the study did not affect the statistical significance of the finding for the primary outcome; the *P* value for difference time in hypoglycemia <70 mg/dL remained <0.05 for SH versus DH.

Secondary Outcomes

The prespecified and ad hoc secondary outcomes are detailed in Table 2. The percentage of time in range (70–180 mg/dL) over the entire observation period was comparable between DH and SH at 71.0% (SD 9.2) and 72.6% (SD 9.2), respectively, with a paired difference SH-DH of 1.6% (−2.1 to 5.2; $P = 0.400$). Figure 2 shows an interquartile plot of glucose values over the entire study duration. The percentage of time in range was significantly lower in PLGS at 63.4% (SD 16.6), representing a difference PLGS-DH of −7.5% ($P = 0.044$). The mean number of rescue carbohydrate treatments per day was lower in DH compared with SH or PLGS, at 0.6 (SD 0.9) vs. 1.7 (SD 2.0) vs. 1.4 (SD 1.3), respectively; the paired ratio difference PLGS-DH was 2.4 ($P = 0.033$) and SH-DH was 3 ($P = 0.003$).

For the entire study duration, the percentage of time in hypoglycemia (<70 mg/dL) was significantly lower for DH compared with SH (0.5% vs. 1.3%, $P = 0.005$) and for DH compared with PLGS (0.5% vs. 1.5%, $P = 0.019$). Also, for the entire study duration, mean glucose was similar between DH and PLGS (159.2 vs. 163.6 mg/dL, $P = 0.360$), while SH had a lower mean glucose (SH 151.6 vs. DH 159.2 mg/dL, $P = 0.002$). Extreme hyperglycemia (>250 mg/dL) and hyperglycemia (>180 mg/dL) were most frequently observed with PLGS (see Table 2).

For the period from exercise start until 4 h after, participants experienced a median time in hypoglycemia (<70 mg/dL) of 0 min in the DH arm vs. 23.7 min for SH arm and 12.0 min for PLGS. With the reduction in hypoglycemia, there was an increase in the median time in hyperglycemia (>180 mg/dL) during the exercise period (DH 20.8% vs. SH 6.3% vs. PLGS 4.2%; PLGS-DH $P = 0.015$ and SH-DH $P = 0.038$) along with a higher mean glucose (DH 146.3 vs. SH 124.4 vs. PLGS 123.2 mg/dL).

Overnight (12:00 A.M.–6:00 A.M.) glycemic metrics including time in range (70–180 mg/dL), hyperglycemia (>180 mg/dL), and extreme hyperglycemia (>250 mg/dL) were similar for SH and DH. The closed-loop systems both showed improved time in range and lower mean glucose for the overnight period compared with the control system, PLGS (see Table 2).

The average daily amount of insulin delivered did not differ across the study arms (DH 42.3, SH 41.1, PLGS 44.0 units/day, all $P = NS$). Mean glucagon use per day in the DH arm was 364.5 μg/day (SD 4.5). More glucagon was delivered on day 1 that included the structured in-clinic exercise session (mean glucagon delivery: day 1, 551; day 2, 290, and day 3, 311 μg). See Supplementary Fig. 4 for

insulin and glucagon delivery in the full study. See Supplementary Fig. 5 for detailed view of insulin and glucagon delivery during the in-clinic exercise session.

While the liquid stable glucagon was generally tolerated by the study participants, five participants (23%) experienced nausea during the DH arm (four of these were deemed related to glucagon and one was due to viral gastroenteritis), two participants (9%) had emesis, and two participants (9%) had headache. One participant had burning at the glucagon infusion site (5%). Two participants (9%) had slight edema at the glucagon pod site, and six participants (27%) had slight erythema at the glucagon pod site at the time of study completion. All adverse events were mild or moderate in severity, self-limited, and required no intervention. Discomfort with glucagon administration was assessed using a visual analog score on day 1 and day 4, where a score of 0 represented no discomfort and a score of 100 represented worst discomfort. The mean visual analog score was 29.1 (SD 26.4), with no significant difference between scores on day 1 and day 4.

Adverse Events

There were no serious adverse events in any of the treatment groups.

CONCLUSIONS

We present results on a DH closed-loop system that uses liquid stable glucagon to help prevent hypoglycemia during and after exercise in people with type 1 diabetes. Currently available intramuscular glucagon kits are U.S. Food and Drug Administration approved only for immediate use after reconstitution. Use of a liquid stable form of glucagon eliminated the need for pump reservoir changes every 24 h. There was self-limited, mild to moderate infusion site discomfort for some participants. No participants experienced a serious adverse event. Future work is needed to determine whether this discomfort can be mitigated by further adjustments to the dosage and rate of glucagon delivery. The safety of long-term glucagon dosing has been demonstrated in animals (29), but still needs to be established in humans. This is a critical step for DH closed-loop systems to be successfully commercialized.

The use of DH closed-loop systems to reduce hypoglycemia has been published by others (2,5,15). There are DH systems under commercial development, such as the iLet (Beta Bionics, Boston, MA). Taleb et al. (2) demonstrated an improvement in hypoglycemia with a DH system compared with an SH system when exercise was announced 20 min before exercise. Abitbol et al. (5) demonstrated that a DH and SH system both prevented nocturnal hypoglycemia in participants with hypoglycemia unawareness. Exercise was not included as part of their study. Two recent studies of insulin-only closed-loop systems included exercise. In Ekhlaspour et al. (30), adolescents and children participated in prolonged intense exercise at a winter ski camp with 0% time in hypoglycemia $<$70 mg/dL during skiing in both control and intervention groups. Forlenza et al. (31) reported 1.4–1.6% time in hypoglycemia $<$70 mg/dL for the 12 h after exercise. This study included $\geq$30 min of moderate-intensity exercise that included both aerobic and mixed aerobic/anaerobic activities and allowed proactive carbohydrate intake before exercise. As expected, based on the nature and timing of exercise and the preexercise meals/carbohydrate intake, these two studies showed a lower proportional time in hypoglycemia than our current study.

SH systems have also been developed to reduce exercise-related hypoglycemia using heart rate to detect exercise and adjust insulin dosing (32). An SH system was used to successfully control glucose in adolescents with type 1 diabetes while skiing and snowboarding (33). Our previous outpatient study (1) demonstrated that the use of glucagon in a DH closed-loop system with automated exercise detection significantly reduced hypoglycemia in active adults with type 1 diabetes. In that study, however, the addition of glucagon reduced but did not eliminate hypoglycemia. In the study described here, we aimed to further reduce hypoglycemia with the use of a hypoglycemia-prediction algorithm at the start of exercise, combined with the delivery of a minidose of glucagon in response to predicted hypoglycemia.

For the in clinic exercise period, the DH closed-loop system described here significantly reduced the need for rescue carbohydrates and hypoglycemia ($<$70 mg/dL) compared with the SH system, significantly reduced the need for rescue carbohydrates compared with the PLGS system, and nearly eliminated clinically important hypoglycemia ($<$54 mg/dL). When considering the hypoglycemia outcomes in this study, it is important to note that the study protocol in all treatment arms required prompt treatment with 15 g carbohydrate of any glucose values $<$70 mg/dL, which does not necessarily reflect real-world treatment of hypoglycemia. Longer studies in free-living participants are needed to evaluate these hypoglycemia outcomes.

However, this reduction in hypoglycemia came at the cost of increased hyperglycemia in the exercise period (see Table 2 and Fig. 1). Four participants experienced glucagon-related nausea, and three of these participants withdrew related to this complaint. Although there are some considerations that may minimize nausea, this adverse effect is a potential limitation for DH therapy in real-world use. One of the limitations of this study was that a fixed amount of minidose glucagon was administered. In the future, adapting the preexercise glucagon dose to a larger or smaller amount based on the person's risk of hypoglycemia may be more successful and mitigate the risk of nausea.

No blinding to the use of glucagon was used in this study, which limits the full assessment of adverse effects (pain, nausea, etc.) and did allow for possible bias of participant behavioral change. The rates of nausea seen in this study are comparable to other recent studies; El-Khatib et al. (9) reported 21 of 39 participants (53%) with nausea during an 11-day DH closed-loop study. In a pharmacokinetic/dynamic study by Hövelmann et al. (34), double-blinded dosing of dasiglucagon and lyophilized glucagon resulted in nausea in up to 10% of participants at dose of 30 μg and in up to 50% with dose of 600 μg.

By chance, mean glucose at the start of the study was higher in the DH arm than in SH or PLGS. However, after adjusting for the higher glucose in DH at the start of the experiment, the primary outcome of percentage time in hypoglycemia $<$70 mg/dL remained statistically significant for the difference between DH and SH. The DH system is designed to dose glucagon throughout the day when glucose is trending low. Notably, before the start of in-clinic exercise, eight subjects had already received glucagon (ranging from 67.5 to 155.0 μg). The higher glucose before exercise in the DH hormone arm may be partially explained by the glucagon given before the start of exercise. This

study was not designed to assess how glucagon given before exercise may impact the glucose response during exercise.

Comparisons between SH and DH are exploratory only because this study was not powered for this comparison. For the full study, the times in range for the SH and DH arms were very similar. The algorithm used to calculate insulin amount is identical in both the DH and SH control algorithms. The time in range for our SH system is similar to other insulin-only closed-loop systems, although differences in study design make direct comparisons difficult (35–38). The main advantage of the DH system is less hypoglycemia with the tradeoff of slightly more hyperglycemia. Time in range and mean glucose could be improved in a DH system with more aggressive insulin delivery relying on glucagon delivery to treat hypoglycemia. We have opted against this approach for safety due to the risk of hypoglycemia if glucagon fails to deliver. For the full study, the median time of $<$70 mg/dL was 11.5 min/day lower for DH compared with SH and 23.7 min lower during the exercise period. The trend toward lower time in hypoglycemia holds true for the full study period when excluding the exercise plus 4-h period (mean $<$70 mg/dL: DH 1.0%, SH 2.3%, PLGS 1.9%). For the full study, the median time of $>$180 mg/dL was lowest for SH at 361.4 min/day, followed by DH, which was 406.1 min/day, and then PLGS, which was 499.7 min/day. On the basis of the higher cost and increased complexity required of a DH system, DH closed-loop control may not be for all users but could provide additional protection against exercise-induced hypoglycemia and may appeal to those who experience frequent hypoglycemia.

Another study, by Rickels et al. (39), showed that supplying study participants with minidose glucagon before exercise could help eliminate hypoglycemia. While Rickels et al. were able to completely eliminate exercise-related hypoglycemia using minidoses of glucagon before exercise, our automated system was not able to do so. One explanation for this is that the participants in the Rickels et al. (39) study were exercising in the fasted state when insulin-on-board was at its lowest level of the day. Participants in the current study were exercising 2 h after lunch, during a time of nearly maximal insulin-on-board. It may be that during this period of time, more glucagon may be necessary to prevent exercise-induced hypoglycemia, although a higher dose may be less well tolerated. When possible, exercise should generally be undertaken when insulin-on-board is low. While a DH control system combined with a predicted hypoglycemia algorithm may substantially reduce hypoglycemia, it will likely not eliminate it completely when there is a significant amount of insulin-on-board.

Another limitation of this study was that the in-clinic exercise was limited to aerobic exercise. Glycemic response to exercise varies based on exercise type, duration, and intensity (19). Participants were free to perform other types of exercise during the outpatient portion of the study. The hypoglycemia-prediction algorithm used in this study was specific to aerobic exercise. Further work is needed to expand this prediction algorithm to include other types of exercise as well as to take into account other features such as intensity and duration of exercise. Automated detection of exercise reduces burden on the person with diabetes; however, this delays the insulin algorithm adjustments until the exercise threshold is reached. A limitation is that we were not able to capture events when the study participants were exercising but the exercise detection algorithm did not trigger (a false-negative exercise detection), thereby requiring the user to announce to the system that they were exercising. Also, we did not collect detailed information on early pod failures. Another limitation of this study is that we excluded people with hypoglycemia unawareness or a recent history of severe hypoglycemia. Further work is needed to study this high-risk population, who may greatly benefit from automated glucagon delivery.

In summary, we demonstrated that a DH closed-loop system significantly reduced hypoglycemia and rescue carbohydrate treatments during and after aerobic exercise and demonstrated feasibility of automated liquid stable glucagon delivery in a short, open-label study. Optimization of glucagon dose and timing in future studies may minimize hyperglycemia and nausea.

Funding and Duality of Interest. This study was sponsored by Xeris Pharmaceuticals via a subaward from JDRF (Industry Discovery & Development Partnerships grant #15-2013-505). P.G.J. and J.R.C. have a financial interest in Pacific Diabetes Technologies, Inc., a company that may have a commercial interest in the results of this type of research and technology. This potential conflict of interest has been reviewed and managed by OHSU. In addition, P.G.J. and J.R.C. report research support from Xeris, Dexcom, and Tandem Diabetes Care. J.R.C. reports advisory board participation for Zealand Pharma, Novo Nordisk, and AstraZeneca, consulting for Dexcom, and a U.S. patent on the use of ferulic acid to stabilize glucagon. No other potential conflicts of interest relevant to the article were reported.

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Author Contributions. L.M.W., P.G.J., J.E.Y., and J.R.C. contributed to the writing, literature search, study design, data collection, data analysis, data interpretation, closed-loop system construction, and figures. K.L.R. contributed to the statistical analysis and tables. N.R., R.R., and J.L. contributed to data collection and closed-loop system construction. D.B., V.G., F.G., and B.S. contributed to the data collection and tables. I.I.K.S. and N.S.T. contributed to the statistical analysis and figures. L.M.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 79th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 7–11 June 2019.

References

1. Castle JR, El Youssef J, Wilson LM, et al. Randomized outpatient trial of single- and dual-hormone closed-loop systems that adapt to exercise using wearable sensors. Diabetes Care 2018;41:1471–1477

2. Taleb N, Emami A, Suppere C, et al. Efficacy of single-hormone and dual-hormone artificial pancreas during continuous and interval exercise in adult patients with type 1 diabetes: randomised controlled crossover trial. Diabetologia 2016;59:2561–2571

3. Jacobs PG, El Youssef J, Reddy R, et al. Randomized trial of a dual-hormone artificial pancreas with dosing adjustment during exercise compared with no adjustment and sensor-augmented pump therapy. Diabetes Obes Metab 2016;18:1110–1119

4. Haidar A, Legault L, Messier V, Mitre TM, Leroux C, Rabasa-Lhoret R. Comparison of dual-hormone artificial pancreas, single-hormone artificial pancreas, and conventional insulin pump therapy for glycaemic control in patients with type 1 diabetes: an open-label randomised controlled crossover trial. Lancet Diabetes Endocrinol 2015;3:17–26

5. Abitbol A, Rabasa-Lhoret R, Messier V, et al. Overnight glucose control with dual- and single-hormone artificial pancreas in type 1 diabetes with hypoglycemia unawareness: a randomized controlled trial. Diabetes Technol Ther 2018;20:189–196

6. El-Khatib FH, Russell SJ, Nathan DM, Sutherlin RG, Damiano ER. A bihormonal closed-loop artificial pancreas for type 1 diabetes. Sci Transl Med 2010;2:27ra27

7. Russell SJ, El-Khatib FH, Sinha M, et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. N Engl J Med 2014;371:313–325

8. van Bon AC, Luijf YM, Koebrugge R, Koops R, Hoekstra JB, DeVries JH. Feasibility of a portable bihormonal closed-loop system to control glucose excursions at home under free-living conditions for 48 hours. Diabetes Technol Ther 2014; 16:131–136
9. El-Khatib FH, Balliro C, Hillard MA, et al. Home use of a bihormonal bionic pancreas versus insulin pump therapy in adults with type 1 diabetes: a multicentre randomised crossover trial. Lancet 2017;389:369–380
10. Russell SJ, Hillard MA, Balliro C, et al. Day and night glycaemic control with a bionic pancreas versus conventional insulin pump therapy in preadolescent children with type 1 diabetes: a randomised crossover trial. Lancet Diabetes Endocrinol 2016;4:233–243
11. Haidar A, Legault L, Dallaire M, et al. Glucose-responsive insulin and glucagon delivery (dual-hormone artificial pancreas) in adults with type 1 diabetes: a randomized crossover controlled trial. CMAJ 2013;185:297–305
12. Haidar A, Legault L, Matteau-Pelletier L, et al. Outpatient overnight glucose control with dual-hormone artificial pancreas, single-hormone artificial pancreas, or conventional insulin pump therapy in children and adolescents with type 1 diabetes: an open-label, randomised controlled trial. Lancet Diabetes Endocrinol 2015;3:595–604
13. Haidar A, Rabasa-Lhoret R, Legault L, et al. Single- and dual-hormone artificial pancreas for overnight glucose control in type 1 diabetes. J Clin Endocrinol Metab 2016;101:214–223
14. Gingras V, Rabasa-Lhoret R, Messier V, Ladouceur M, Legault L, Haidar A. Efficacy of dual-hormone artificial pancreas to alleviate the carbohydrate-counting burden of type 1 diabetes: a randomized crossover trial. Diabetes Metab 2016;42:47–54
15. Haidar A, Messier V, Legault L, Ladouceur M, Rabasa-Lhoret R. Outpatient 60-hour day-and-night glucose control with dual-hormone artificial pancreas, single-hormone artificial pancreas, or sensor-augmented pump therapy in adults with type 1 diabetes: an open-label, randomised, crossover, controlled trial. Diabetes Obes Metab 2017;19:713–720
16. Castle JR, Engle JM, El Youssef J, et al. Novel use of glucagon in a closed-loop system for prevention of hypoglycemia in type 1 diabetes. Diabetes Care 2010;33:1282–1287
17. Van Bon AC, Jonker LD, Koebrugge R, Koops R, Hoekstra JB, DeVries JH. Feasibility of a bihormonal closed-loop system to control post-exercise and postprandial glucose excursions. J Diabetes Sci Technol 2012;6:1114–1122
18. Blauw H, van Bon AC, Koops R, DeVries JH; on behalf of the PCDIAB consortium. Performance and safety of an integrated bihormonal artificial pancreas for fully automated glucose control at home. Diabetes Obes Metab 2016;18:671–677
19. Riddell MC, Gallen IW, Smart CE, et al. Exercise management in type 1 diabetes: a consensus statement. Lancet Diabetes Endocrinol 2017;5:377–390
20. Wilson LM, Castle JR. Stable liquid glucagon: beyond emergency hypoglycemia rescue. J Diabetes Sci Technol 2018;12:847–853
21. Jacobs PG, El Youssef J, Castle J, et al. Automated control of an adaptive bihormonal, dual-sensor artificial pancreas and evaluation during inpatient studies. IEEE Trans Biomed Eng 2014;61:2569–2581
22. Zhong A, Choudhary P, McMahon C, et al. Effectiveness of automated insulin management features of the MiniMed® 640G sensor-augmented insulin pump. Diabetes Technol Ther 2016;18:657–663
23. Jacobs PG, Resalat N, El Youssef J, et al. Incorporating an exercise detection, grading, and hormone dosing algorithm into the artificial pancreas using accelerometry and heart rate. J Diabetes Sci Technol 2015;9:1175–1184
24. Reddy R, Resalat N, Wilson LM, Castle JR, El Youssef J, Jacobs PG. Prediction of hypoglycemia during aerobic exercise in adults with type 1 diabetes. J Diabetes Sci Technol 2019;13:919–927
25. Resalat N, El Youssef J, Tyler N, Castle J, Jacobs PG. A statistical virtual patient population for the glucoregulatory system in type 1 diabetes with integrated exercise model. PLoS One 2019; 14:e0217301
26. Resalat N. El Youssef J, Reddy R, Castle J, Jacobs PG. Adaptive tuning of basal and bolus insulin to reduce postprandial hypoglycemia in a hybrid artificial pancreas. J Process Contr 2019; 80:247–254
27. Schulz KF, Grimes DA. Multiplicity in randomised trials I: endpoints and treatments. Lancet 2005;365:1591–1595
28. StataCorp LLC. Stata Statistical Software, College Station, TX, StataCorp LLC, 2017
29. Castle JR, Elander M. Long-term safety and tolerability of dasiglucagon, a stable-in-solution glucagon analogue. Diabetes Technol Ther 2019; 21:94–96
30. Ekhlaspour L, Forlenza GP, Chernavvsky D, et al. Closed loop control in adolescents and children during winter sports: use of the Tandem Control-IQ AP system. Pediatr Diabetes 2019;20: 759–768
31. Forlenza GP, Buckingham BA, Christiansen MP, et al. Performance of Omnipod personalized model predictive control algorithm with moderate intensity exercise in adults with type 1 diabetes. Diabetes Technol Ther 2019;21:265–272
32. DeBoer MD, Cherñavvsky DR, Topchyan K, Kovatchev BP, Francis GL, Breton MD. Heart rate informed artificial pancreas system enhances glycemic control during exercise in adolescents with T1D. Pediatr Diabetes 2017;18:540–546
33. Breton MD, Cherñavvsky DR, Forlenza GP, et al. Closed-loop control during intense prolonged outdoor exercise in adolescents with type 1 diabetes: the artificial pancreas ski study. Diabetes Care 2017;40:1644–1650
34. Hövelmann U, Olsen MB, Mouritzen U, Lamers D, Kronshage B, Heise T. Low doses of dasiglucagon consistently increase plasma glucose levels from hypoglycaemia and euglycaemia in people with type 1 diabetes mellitus. Diabetes Obes Metab 2019;21:601–610
35. Bekiari E, Kitsios K, Thabit H, et al. Artificial pancreas treatment for outpatients with type 1 diabetes: systematic review and meta-analysis. BMJ 2018;361:k1310
36. Brown SA, Kovatchev BP, Raghinaru D, et al.; iDCL Trial Research Group. Six-month randomized, multicenter trial of closed-loop control in type 1 diabetes. N Engl J Med 2019;381:1707–1717
37. Haidar A, Tsoukas MA, Bernier-Twardy S, et al. A novel dual-hormone insulin-and-pramlintide artificial pancreas for type 1 diabetes: a randomized controlled crossover trial. Diabetes Care 2020;43:597–606
38. Weisman A, Bai JW, Cardinez M, Kramer CK, Perkins BA. Effect of artificial pancreas systems on glycaemic control in patients with type 1 diabetes: a systematic review and meta-analysis of outpatient randomised controlled trials. Lancet Diabetes Endocrinol 2017;5:501–512
39. Rickels MR, DuBose SN, Toschi E, et al.; T1D Exchange Mini-Dose Glucagon Exercise Study Group. Mini-dose glucagon as a novel approach to prevent exercise-induced hypoglycemia in type 1 diabetes. Diabetes Care 2018;41:1909–1916