Novel Use of Glucagon in a Closed-Loop System for Prevention of Hypoglycemia in Type 1 Diabetes

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OBJECTIVE — To minimize hypoglycemia in subjects with type 1 diabetes by automated glucagon delivery in a closed-loop insulin delivery system.

RESEARCH DESIGN AND METHODS — Adult subjects with type 1 diabetes underwent one closed-loop study with insulin plus placebo and one study with insulin plus glucagon, given at times of impending hypoglycemia. Seven subjects received glucagon using high-gain parameters, and six subjects received glucagon in a more prolonged manner using low-gain parameters. Blood glucose levels were measured every 10 min and insulin and glucagon infusions were adjusted every 5 min. All subjects received a portion of their usual premeal insulin after meal announcement.

RESULTS — Automated glucagon plus insulin delivery, compared with placebo plus insulin, significantly reduced time spent in the hypoglycemic range (15 ± 6 vs. 40 ± 10 min/day, $P = 0.04$). Compared with placebo, high-gain glucagon delivery reduced the frequency of hypoglycemic events (1.0 ± 0.6 vs. 2.1 ± 0.6 events/day, $P = 0.01$) and the need for carbohydrate treatment (1.4 ± 0.8 vs. 4.0 ± 1.4 treatments/day, $P = 0.01$). Glucagon given with low-gain parameters did not significantly reduce hypoglycemic event frequency (P = NS) but did reduce frequency of carbohydrate treatment ($P = 0.05$).

CONCLUSIONS — During closed-loop treatment in subjects with type 1 diabetes, high-gain pulses of glucagon decreased the frequency of hypoglycemia. Larger and longer-term studies will be required to assess the effect of ongoing glucagon treatment on overall glycemic control.

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Severe hypoglycemia is an acute complication of insulin therapy that can lead to seizures, coma, and death (1) and creates a barrier to optimal glycemic control in diabetes management (2). Despite treatment advances such as insulin pump therapy and continuous glucose monitoring, hypoglycemia remains a concern, even when insulin is given in a closed-loop system (3). Here, we report on a novel, automated, sensor-controlled method of insulin delivery accompanied by glucagon delivery at times of impending hypoglycemia.

A closed-loop system consists of a glucose-measuring device, from which data are collected and entered into an algorithm, which in turn controls insulin delivery (4). The difficulty of delivering regular or analog insulin in such a manner is related to its slow onset and prolonged effect when delivered subcutaneously. Until a more rapidly acting insulin preparation is available, discontinuation of subcutaneous insulin during impending hypoglycemia, with any algorithm, may be insufficient to prevent hypoglycemia.

Glucagon, a hormone secreted from the α-cells of the normal endocrine pancreas, rapidly raises circulating glucose levels within minutes via glycogenolysis, even when given subcutaneously (5). Glucagon is approved for use as a parenteral injection for treatment of severe hypoglycemia. In children, an off-label use has been described using small subcutaneous doses to prevent or treat mild hypoglycemia (6,7).

In 1982, Shichiri et al. (8) published the concept of including glucagon delivery in an automated closed-loop glycemic control system. More recently, such a system has been studied in animals by our group (9) and by the Boston University group (10) with promising results. In this study of subjects with type 1 diabetes, we compared the frequency and duration of hypoglycemia during treatment with insulin plus glucagon to treatment with insulin plus placebo. Delivery of insulin and glucagon was automated and controlled by an amperometric glucose sensor. We hypothesized that when given for impending hypoglycemia, glucagon would decrease the frequency of overt hypoglycemia more than placebo.

RESEARCH DESIGN AND METHODS

— Patients were recruited from the Oregon Health and Science University (OHSU) outpatient clinics in Portland, Oregon. Patients who were pregnant or had cardiovascular, cerebrovascular, kidney, or liver disease or any other uncontrolled chronic medical conditions were excluded. Other exclusion criteria included oral or parenteral corticosteroid use, immunosuppressant use, visual or physical impairments that impede the use of a continuous glucose-monitoring device, insulin or glucagon allergy, hypoglycemia unawareness or hospitalization within the past 2 years for severe hypoglycemia, serum insulin antibody titer >100 μU/ml, or requirement of >200 units insulin/day. The research protocol was approved by the OHSU Institutional Review Board, and all subjects provided written informed consent. Per-

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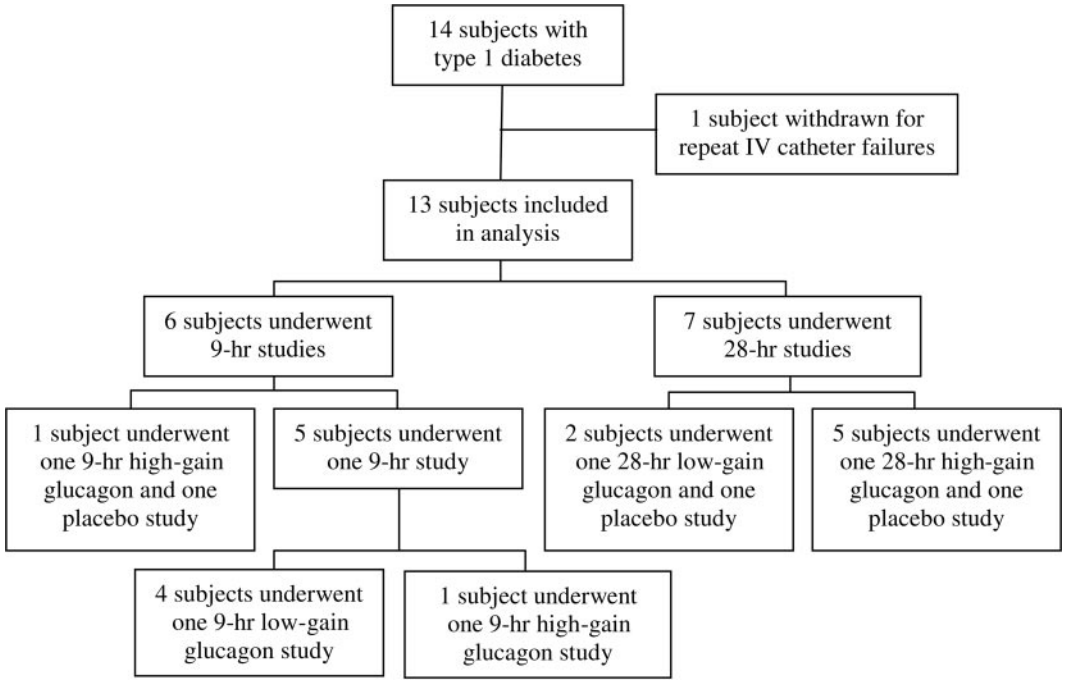

Figure 1—*Study diagram depicting the number of subjects studied under each condition and the study lengths.*

mission to carry out these studies was granted by the U.S. Food and Drug Administration (FDA) (investigational device exemption no. G080130).

A total of 22 closed-loop studies in 14 subjects were performed. Age was 36.7 ± 3.7 years, with a duration of diabetes of 14.1 ± 3.1 years. A1C was 7.6 ± 0.3% and BMI 27.8 ± 1.5 kg/m^2. The study for one patient was stopped early because of repeated intravenous catheter failures. The data from this study were excluded from the analysis, leaving 21 datasets from 13 subjects.

As requested by the FDA, five subjects participated in single 9-h studies with both insulin and glucagon to assess the safety and effectiveness of the study protocol. Eight subjects underwent one study with insulin and placebo and one with insulin and glucagon (see Fig. 1). Of the 13 studies during which glucagon was given, it was delivered using high-gain parameters in seven studies and using low-gain parameters in six. Low- versus high-gain glucagon is discussed in detail below. The treatment order of each paired study was determined by a randomization scheme. In paired studies, subjects were blinded as to whether they received glucagon or placebo.

Subjects wore two subcutaneous glucose sensors, either DexCom Seven Plus or Medtronic Guardian Real-Time glucose sensors. Sensors were placed 8–24 h prior to beginning the study. For subjects taking long-acting insulin at night, the dose was reduced by 50% the night prior to the study. The following morning, subjects were admitted to the Oregon Clinical and Translational Research Institute at OHSU. An intravenous catheter was placed in a forearm vein. The forearm was warmed with a heating pad to arterialize the venous blood. Venous glucose was measured every 10 min in duplicate using a HemoCue Glucose 201 Analyzer. Glucose sensor readings were recorded from the receivers every 5 min. For the first 2 h, the insulin and glucagon delivery rates were determined by venous glucose levels. After the first 2 h, the sensed glucose values from the sensor with better accuracy were input into the algorithm every 5 min to determine the hormone delivery rates. If the sensor accuracy became suboptimal, defined as a median absolute relative difference (MARD) exceeding 20% or median absolute difference (MAD) exceeding 20 mg/dl, control was switched to the other sensor. If the accuracy of both sensors was poor, control was switched to venous glucose and the sensors were recalibrated. Sensors were calibrated at a minimum of every 12 h.

The Fading Memory Proportional Derivative (FMPD) algorithm (9,11) was used to determine the insulin and subcutaneous glucagon (or placebo) delivery rates. Aspart insulin (Novo Nordisk) was delivered subcutaneously via an Animas IR 1000 insulin pump. Glucagon or saline placebo was given through a subcutaneous catheter via a Medfusion 2001 syringe pump. One milligram of glucagon (Novo Nordisk) was mixed with 3 ml of sterile water. The glucagon preparation was freshly reconstituted every 8 h. A study physician was onsite at all times and had the ability to override the hormone infusion rates called for by the FMPD algorithm, which occurred only 1.7% of the time. Either a registered nurse or physician was responsible for adjusting the insulin delivery rate and glucagon delivery rate every 5 min, based on the controller output.

The FMPD algorithm determined the hormone delivery rates based on proportional error, defined as the difference between the current glucose level and the target level, and the derivative error, defined as the rate of change of the glucose. The "fading memory" designation refers to weighting recent errors more heavily than remote errors. This weighting provides an adaptive component to the algorithm, as described previously (9,11). In simple terms, the insulin rate was increased for high or rising glucose levels and glucagon was given for low or falling glucose levels. The basal insulin infusion rate (in units per hour) was given at a rate of 35% of the patient's typical total daily insulin dose, divided by 24.

Determination of insulin delivery

In the FMPD algorithm, the gain factors determined the degree to which proportional or derivative errors led to changes in hormone delivery rates. There were separate gain factors for insulin and glucagon. Positive proportional errors (glucose level above target) and positive derivative errors (rising glucose level) called for an increase in the insulin delivery rate. The overall insulin delivery rate was determined by adding the rates called for by the proportional error (IIR_{pe}), the derivative error (IIR_{de}), and the basal insulin rate.

The proportional error gain factor was $1.2 \times 10^{-3} \pm 0.078 \times 10^{-3}$ units/kg per mg/dl/h for glucagon studies and 1.3×10^{-3} units/kg per mg/dl/h for placebo studies. The derivative error gain factor was $2.0 \times 10^{-3} \pm 0.096 \times 10^{-3}$ units/kg per mg/dl for glucagon studies and was 2.0×10^{-3} units/kg per mg/dl for placebo studies. The mean blood glucose target was 110 ± 1 mg/dl for glucagon studies and 110 mg/dl for placebo studies. There were no significant differences between any of these parameters between the groups. For subjects who underwent two closed-loop studies, the algorithm parameters were identical for both.

Insulin on board, the amount of insulin that had been delivered and was assumed to be active, was continually estimated using a model that we derived from data published by Holmes et al. (12). To minimize hypoglycemia, the insulin infusion was discontinued if the estimated insulin on board reached 15% of the subject's estimated total daily insulin requirement.

Determination of glucagon delivery

The proportional and derivative error gain factors for glucagon were negative, such that negative proportional and derivative errors called for an increase in the glucagon rate. For glucagon, the average weighted proportional error was calculated over a 15 min interval and the average weighted derivative error was calculated over a 10 min interval. There was no basal glucagon infusion rate.

In this project, we tested two closely related algorithms for administering glucagon. Four subjects completed 9-h studies and two subjects completed 28-h studies with low-gain factor settings. In these low-gain glucagon studies, the mean proportional error gain factor was −0.23 ± 0.04 ml/kg per mg/dl/h, the mean derivative error gain factor was −0.06 ± 0.009 ml/kg per mg/dl, and target glucose for glucagon infusion was 108 ± 3 mg/dl. Two subjects completed 9-h studies and five subjects completed 28-h studies with high-gain factor settings. For all of these high-gain glucagon studies, the proportional error gain factor was −2.70 ml/kg per mg/dl/hour, the derivative gain factor was −0.60 ml/kg per mg/dl, and the target glucose for glucagon infusion was 97 ± 1 mg/dl. To avoid overdelivery of glucagon, when total glucagon delivery over the prior 50 min reached a ceiling of 1.0 μg/kg, the algorithm initiated a refractory period for the subsequent 50 min, during which glucagon could not be delivered. Thus, short pulses of glucagon delivery over 5–10 min were followed by the absence of glucagon delivery for 50 min. The insulin rate was reduced by 75% for 40 min after each maximal glucagon pulse.

Meals

Patients were given two meals during each 9-h study and four meals during each 28-h study. Each meal was announced to the controller and an open loop premeal bolus was given. Aspart insulin was given 0–10 min before meals, depending on the subject's premeal glucose level. For low-gain glucagon studies, 53.3 ± 7.0% of usual premeal insulin dose was given. The amount of premeal insulin was increased after the first four studies because of a pattern of postprandial hyperglycemia in those studies. For all placebo and high-gain glucagon studies, 75% of the usual premeal insulin dose was given.

Hypoglycemic treatment

Subjects were treated for hypoglycemia if the venous glucose value fell below 70 mg/dl. For glucose levels 60–69 mg/dl, subjects were given 15 g oral carbohydrate, and the treatment repeated as needed every 15 min. For a glucose value <60 mg/dl, 10 g dextrose was given intravenously.

Statistical analysis

Arterialized venous glucose values, not sensed glucose values, were used to compare hypoglycemia and glucose control between groups. Glucose area under the curve (AUC) was calculated as published elsewhere (13). Minutes in the hypoglycemic range, defined as glucose <70 mg/dl, hypoglycemic events, treatments for hypoglycemia, units of insulin delivered, and micrograms of glucagon delivered were normalized to 24 h for data from both 9- and 28-h studies. Data are expressed as means ± SE. Sensor accuracy was calculated by comparing sensor glucose to reference glucose values (14). Comparisons were made using paired or unpaired *t* tests, as appropriate. Calculations were performed using Microsoft Excel 2007 (version 12).

RESULTS — Six women and seven men with type 1 diabetes participated in a total of 21 human closed-loop studies with a duration of 21.5 ± 2.0 h. Seven subjects received glucagon delivered in a brisk fashion (high-gain) and six subjects received glucagon delivered in a slower fashion (low-gain). In both the high- and low-gain glucagon studies, glucagon was typically delivered at times of impending hypoglycemia when glucose was 90–120 mg/dl, depending on the rate of glucose decline (Fig. 2). At these times, insulin delivery was also markedly reduced or discontinued by the insulin algorithm.

The high-gain glucagon results (paired analysis), low-gain glucagon results (unpaired analysis), and combined high- and low-gain glucagon results (unpaired analysis) are presented separately below. One subject who received high-gain glucagon but did not return for a placebo study was included in the combined results but was not included in the paired high-gain analysis.

High-gain glucagon results

In six subjects who underwent both a high-gain glucagon study and a placebo study, there was a 56% reduction in time spent in the hypoglycemic range (18 ± 11 vs. 41 ± 13 min/day, $P = 0.01$). The number of hypoglycemic events, with events lasting >20 min being considered a new event, was also significantly reduced during the high-gain glucagon versus placebo studies (1.0 ± 0.6 vs. 2.1 ± 0.6 events/day, $P = 0.01$), as was the number of oral or intravenous carbohydrate treatments for hypoglycemia (1.4 ± 0.8 vs. 4.0 ± 1.4 treatments/day, $P = 0.01$). There was no significant difference in mean glucose between the high-gain glucagon versus placebo studies (138 ± 17 vs. 131 ± 17 mg/dl, P = NS), as shown in Fig. 3*A*. The mean fasting glucose was also quite similar (123 ± 14 vs. 120 ± 15 mg/dl, P = NS). There was a nonsignificant trend toward a higher postprandial glucose in high-gain glucagon versus placebo studies, defined as mean value

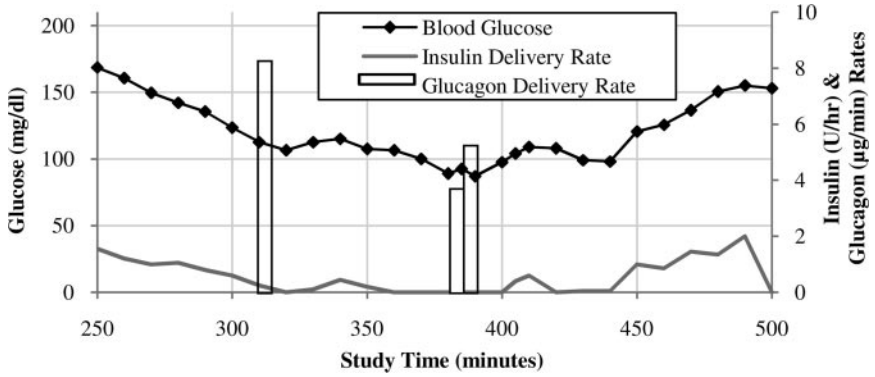

Figure 2—*Example of data taken from a closed-loop study. Venous blood glucose is noted by black diamonds, insulin delivery rate by a gray line, and glucagon delivery rate by rectangles. Note that glucagon is delivered by algorithm in the late postprandial period at times of impending hypoglycemia. Overt hypoglycemia is avoided without the use of carbohydrate supplementation.*

0–180 min after meals (157 ± 18 vs. 144 ± 17 mg/dl, P = NS). The amount of insulin delivered during the high-gain glucagon versus placebo studies was nearly identical (48.9 ± 6.2 vs. 48.3 ± 5.5 units per day, P = NS).

Low-gain glucagon results

In six subjects who received low-gain glucagon compared with the eight subjects who received placebo, there was a nonsignificant reduction in time in the hypoglycemic range (15 ± 8 vs. 40 ± 10 min/day, P = NS). There was also a trend toward a reduction in the number of hypoglycemic events that did not reach statistical significance (1.4 ± 0.7 vs. 2.3 ± 0.5 events/day, P = NS). There was a reduction in the number of treatments for hypoglycemia in studies with low-gain glucagon of borderline significance (1.0 ± 0.7 vs. 3.9 ± 1.0 treatments/day, P = 0.05). Mean glucose was somewhat higher in low-gain glucagon versus placebo studies (157 ± 24 vs. 135 ± 16 mg/dl, P = 0.04). There was also a trend toward higher fasting glucose in the low-gain glucagon versus placebo studies (137 ± 20 vs. 122 ± 13 mg/dl, P = NS). There was a similar trend, of borderline statistical significance, suggesting a larger elevation in postprandial glucose in the low-gain glucagon versus placebo studies (179 ± 26 vs. 151 ± 18 mg/dl, P = 0.05). There was a nonsignificant difference in insulin delivered in low-gain glucagon versus placebo studies (60.1 ± 14.1 vs. 46.9 ± 5.5 units/day). The mean dose of glucagon delivered during the low-gain glucagon studies was higher than the high-gain glucagon studies but did not reach statistical significance (746 ± 134 vs. 516 ± 108 μg/day, P = NS).

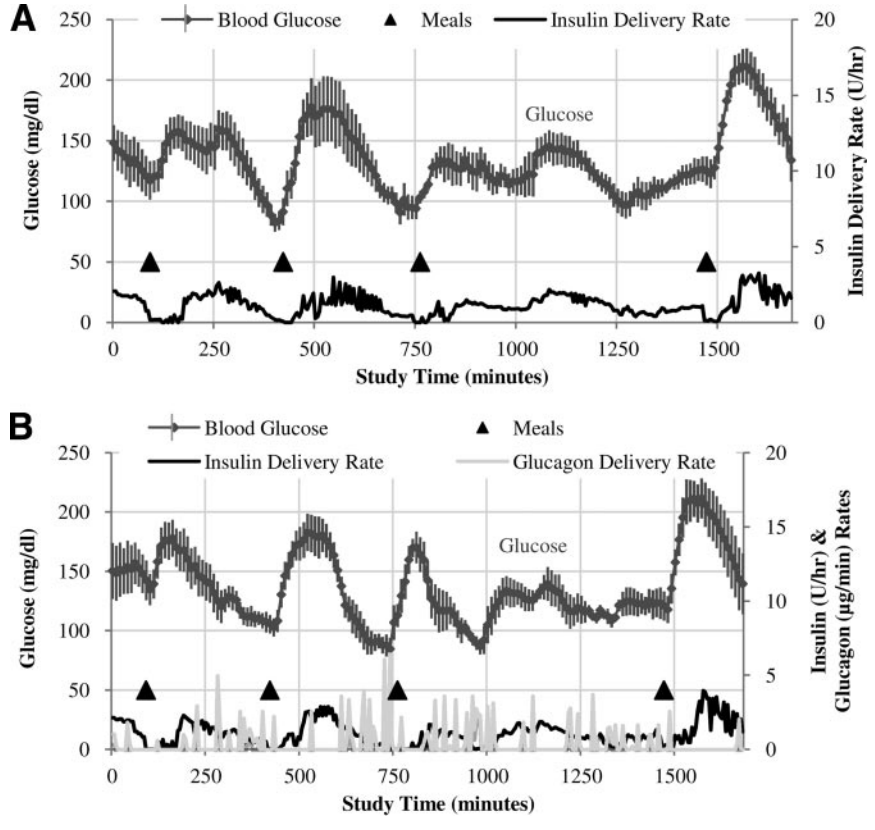

Figure 3—*Summary of glucose levels (means ± SE), insulin delivery rate, and, for glucagon studies, the glucagon delivery rate. Venous blood glucose is noted by gray diamonds, insulin delivery rate by a black line, glucagon delivery rate by a light gray line, and meals by black triangles.* A: *Composite of eight insulin plus placebo studies.* B: *Composite of seven insulin plus high-gain glucagon studies. Insulin delivery and overall glycemic control were similar in both conditions.*

Combined high- and low-gain glucagon results

Glucagon, when given either via high- or low-gain, compared with placebo, led to a 63% reduction of time spent in the hypoglycemic range (15 ± 6 vs. 40 ± 10 min/day, P = 0.04). The number of hypoglycemic events per day was not significantly different between glucagon versus placebo studies (1.1 ± 0.4 vs. 2.3 ± 0.5 events/day, P = NS). The number of treatments for hypoglycemia per day was considerably reduced in the glucagon versus placebo studies (1.1 ± 0.5 vs. 3.9 ± 1.0 treatments/day, P = 0.01). Mean glucose was somewhat higher in the glucagon studies, but this increase did not reach statistical significance (145 ± 14 vs. 135 ± 16 mg/dl, P = NS). Other metrics of glycemic control, including percent of AUC in the target (70–180 mg/dl) and hyperglycemic (>180 mg/dl) ranges and mean amplitude of glycemic excursions were not significantly different between the groups (data not shown).

Sensor accuracy

Overall sensor accuracy was very good, with combined MARD of 8.7 ± 1.5% and MAD of 13.3 ± 1.5 mg/dl. Sensors were calibrated on average every 5.7 ± 0.5 h.

In 8.6% of cases, venous blood, rather than sensed, glucose values were sent to the controller due to suboptimal sensor accuracy.

Tolerability

Only one subject developed transient nausea and vomiting after receiving 350 μg glucagon over 175 min during a low-gain glucagon study. No subjects in the high-gain glucagon or placebo studies experienced any side effects.

CONCLUSIONS — In this automated glycemic control system, we compared the effect of subcutaneous glucagon, delivered in small doses at times of impending hypoglycemia, to saline placebo. In both conditions, the algorithm called for a significant reduction or discontinuation of insulin delivery during impending hypoglycemia. We found that compared with placebo, glucagon delivered in pulses using high-gain parameters significantly decreased the time spent in the hypoglycemic range, the number of hypoglycemic events, and the number of treatments needed for hypoglycemia. Only the high-gain, not the low-gain, glucagon delivery system was superior to placebo in reducing all three of these outcomes, despite the fact that a lower amount of glucagon was delivered in the high-gain studies. The high-gain glucagon infusion consisted of a pulse of glucagon typically given over 5–10 min at a time of impending hypoglycemia followed by a 50-min off period. The low-gain glucagon was delivered in a slow, more prolonged manner without a mandatory off period. The high-gain glucagon infusion is arguably more physiologic, as glucagon is secreted rapidly in response to hypoglycemia in humans without diabetes (15).

Minimizing glucagon delivery, as described here, is important to avoid potential side effects, such as acute hyperglycemia and nausea, and more severe effects, such as depletion of liver glycogen. Notably, the mean glucose levels in the high-gain glucagon and placebo studies were very similar. However, larger and longer-term studies will be required to assess the effect of ongoing glucagon treatment on overall glycemic control.

Limitations of this study include the absence of paired studies for some individuals. In addition, the lower amount of premeal insulin in the low-gain glucagon studies compared with the placebo studies may have affected the results, in particular the differences in mean and postprandial glucose levels. In some regards, the need to announce the meal to the controller and the delivery of substantial amounts of premeal insulin might also be considered a limitation. A true closed-loop system without meal announcement using currently available insulin preparations delivered subcutaneously is unlikely to provide optimal blood glucose control.

After reconstitution, glucagon forms fibrils over time (16,17) and is currently approved for use only immediately after reconstitution. Despite the occurrence of fibrils and aggregates, our group (9) and El-Khatib et al. (18) have shown that even when glucagon is aged for 1 week at room or body temperature, large doses retain full hyperglycemic activity in animals. The reason that the aggregated form of glucagon retains its physiologic effect is unclear. It is possible that, after injection, the aggregates dissociate into monomeric form in the subcutaneous space.

There is some evidence that glucagon can be cytotoxic if it is "aged" at very high concentrations (19), but there are no reports of cytotoxicity during aging at concentrations of 1 mg/ml or lower. Further studies are needed to examine the efficacy of glucagon used for several days after reconstitution and to assess potential cytotoxicity at clinically appropriate concentrations. It is possible that aggregation may be overcome using glucagon analogs (20) or novel methods of glucagon preparation (21).

In conclusion, we found that glucagon given to subjects with type 1 diabetes by algorithm during impending hypoglycemia is effective in preventing most cases of hypoglycemia. Glycemic control was good in this study, in part due to open-loop insulin delivery before meals. These results suggest that an automated system of closed-loop glucagon delivery, with a hybrid pattern of insulin delivery including meal announcement, is able to control glycemia safely and effectively in people with type 1 diabetes. There is a need for further research into the issue of glucagon stability and for the development of a fully automated insulin and glucagon delivery device.

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