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

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Submitted 10 December 2009 and accepted 8 March 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
**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 ten minutes and insulin and glucagon infusions were adjusted every five minutes. All subjects received a portion of their usual pre-meal insulin after meal announcement.

**Results** – Automated glucagon plus insulin delivery, as compared to placebo plus insulin, significantly reduced time spent in the hypoglycemic range (15 ± 6 vs. 40 ± 10 min per day, p = 0.04). Compared to placebo, high gain glucagon delivery reduced the frequency of hypoglycemic events (1.0 ± 0.6 vs. 2.1 ± 0.6 events per day, p = 0.01) and the need for carbohydrate treatment (1.4 ± 0.8 vs. 4.0 ± 1.4 treatments per day, p = 0.01). Glucagon given with low gain parameters did not significantly reduce hypoglycemic event frequency (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.
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 alpha 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 published the concept of including glucagon delivery in an automated closed-loop glycemic control system (8). 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 Oregon Health & Sciences 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 two years for severe hypoglycemia, serum insulin antibody titer over 100 µU/ml, or requirement of greater than 200 units of insulin per day. The research protocol was approved by the OHSU Institutional Review Board and all subjects provided written informed consent. Permission to carry out these studies was granted by the US Food and Drug Administration (Investigational Device Exemption #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. HbA1c was 7.6 ± 0.3% and BMI 27.8 ± 1.5 kg/m². The study for one patient was stopped early in the study because of repeated intravenous (IV) catheter failures. The data from this study were excluded from the analysis, leaving 21 data sets from 13 subjects.
As requested by the FDA, five subjects participated in single 9-hour 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.

**Study procedures** - Subjects wore two subcutaneous glucose sensors, either DexCom Seven Plus or Medtronic Guardian REAL-time glucose sensors. Sensors were placed 8-24 hours 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 & Translational Research Institute at OHSU. An IV 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 ten minutes in duplicate using a HemoCue Glucose 201 Analyzer. Glucose sensor readings were recorded from the receivers every five minutes. For the first two hours, the insulin and glucagon delivery rates were determined by venous glucose levels. After the first two hours, the sensed glucose values from the sensor with better accuracy were input into the algorithm every five minutes 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 hours.

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 mg of glucagon (Novo Nordisk) was mixed with three mL of sterile water. The glucagon preparation was freshly reconstituted every eight hours. A study physician was on site 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 five minutes, 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 per kg per mg/dl per hour for glucagon studies and $1.3 \times 10^{-3}$ for placebo studies. The derivative error gain factor was $2.0 \times 10^{-3} \pm 0.096 \times 10^{-3}$ units per kg per mg/dl for glucagon studies and $2.0 \times 10^{-3}$ for placebo studies. The mean blood glucose target was $110 \pm 1$ mg/dl for glucagon studies and was $110$ 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 minute interval and the average weighted derivative error was calculated over a ten minute interval. There was no basal glucagon infusion rate.

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

### Meals

Patients were given two meals during each 9-hour study and four meals during each 28-hour study. Each meal was announced to the controller and an open loop pre-meal bolus was given. Aspart insulin was given 0-10 minutes before meals, depending on the subject’s pre-meal glucose level. For low gain glucagon studies, $53.3 \pm 7.0\%$ of usual pre-meal insulin dose was given. The amount of pre-meal insulin was increased after the first four studies because of a pattern of post-prandial hyperglycemia in those studies. For all placebo and high gain glucagon studies, $75\%$ of the usual pre-meal 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 grams of oral carbohydrate and treatment repeated as needed every 15
minutes. For a glucose value below 60 mg/dl, 10 grams of dextrose was given IV.

**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) were 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 hours for data from both 9-hr and 28-hr studies. Data are expressed as mean ± SEM. 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 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 hours. 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 (see 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. The 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 the 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 per day, p = 0.01). The number of hypoglycemic events, with events lasting longer than 20 minutes being considered a new event, was also significantly reduced during the high gain glucagon vs. placebo studies (1.0 ± 0.6 vs. 2.1 ± 0.6 events per day, p = 0.01) as was the number of oral or IV carbohydrate treatments for hypoglycemia (1.4 ± 0.8 vs. 4.0 ± 1.4 treatments per day, p = 0.01). There was no significant difference in mean glucose between the high gain glucagon vs. placebo studies (138 ± 17 vs. 131± 17 mg/dl, p = NS), as shown in Fig. 3A. The mean fasting glucose was also quite similar (123 ± 14 vs. 120 ± 15 mg/dl, p = NS). There was a non-significant trend toward a higher post-prandial glucose in high gain glucagon vs. placebo studies, defined as mean value 0-180 minutes after meals (157± 18 vs. 144 ± 17 mg/dl, p = NS). The amount of insulin delivered during the high gain glucagon vs. placebo studies was nearly identical (48.9 ± 6.2 vs. 48.3 ± 5.5 U per day, p = NS).

**Low Gain Glucagon Results** - In the six subjects who received low gain glucagon compared to the eight subjects who received placebo, there was a non-significant reduction in time in the hypoglycemic range (15 ± 8 vs. 40 ± 10 min per 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 per 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 per day, p = 0.05). Mean glucose was somewhat higher in low gain glucagon vs. placebo studies (157 ± 24 vs. 135 ± 16 mg/dl, p =0.04). There was also a trend towards higher fasting glucose in the
low gain glucagon vs. 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 post-prandial glucose in the low gain glucagon vs. placebo studies (179 ± 26 vs. 151 ± 18 mg/dl, p = 0.05). There was a non-significant difference in insulin delivered in low gain glucagon vs. placebo studies (60.1 ± 14.1 vs. 46.9 ± 5.5 U per day). The mean dose of glucagon delivered during the low gain glucagon studies was higher compared to the high gain glucagon studies, but did not reach statistical significance (746 ± 134 vs. 516 ± 108 mcg per day, p = NS).  

**Combined High and Low Gain Glucagon Results** - Glucagon, when given either via high or low gain, as compared to placebo, led to a 63% reduction of time spent in the hypoglycemic range (15 ± 6 vs. 40 ± 10 min per day, p = 0.04). The number of hypoglycemic events per day was not significantly different between glucagon vs. placebo studies (1.1 ± 0.4 vs. 2.3 ± 0.5 events per day, p = NS). The number of treatments for hypoglycemia per day was considerably reduced in the glucagon vs. placebo studies (1.1 ± 0.5 vs. 3.9 ± 1.0 treatments per 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 area under the curve 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 hours. 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 of glucagon over 175 minutes during a low gain glucagon study. No subjects in the high gain glucagon or placebo studies experienced any side effects.  

**DISCUSSION**  
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, as compared to 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 minutes at a time of impending hypoglycemia followed by a 50 minute 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 pre-meal insulin in the low gain glucagon studies compared to the placebo studies may have affected the results, in particular the differences in mean and post-prandial glucose levels. In some regards, the need to announce the meal to the controller and the delivery of substantial amounts of pre-meal 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 one 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 persons 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 persons 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.

ACKNOWLEDGMENTS
This work was supported by grants from the Juvenile Diabetes Research Foundation, Good Samaritan Foundation (Portland, OR), and the National Institutes of Health (NIH) (Grant T32 DK 007674). We thank Jillian Hansen for her technical support. We also thank the staff and research subjects who carried out these studies at the Oregon Clinical and Translational Research Institute (OCTRI), which is supported by grant number UL1 RR024140 from the National Center for Research Resources (NCRR), a component of the NIH, and NIH Roadmap for Medical Research.

The authors have no conflicts of interest to report.
REFERENCES

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

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 post-prandial period at times of impending hypoglycemia. Overt hypoglycemia is avoided without the use of carbohydrate supplementation.

Figure 3 – Summary of glucose levels (mean ± SEM), 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.
Figure 1

14 subjects with type 1 diabetes

1 subject withdrawn for repeat IV catheter failures

13 subjects included in analysis

6 subjects underwent 9-hr studies

1 subject underwent one 9-hr high gain glucagon and one placebo study

5 subjects underwent one 9-hr low gain glucagon study

1 subject underwent one 9-hr high gain glucagon study

7 subjects underwent 28-hr studies

2 subjects underwent one 28-hr low gain glucagon and one placebo study

5 subjects underwent one 28-hr high gain glucagon and one placebo study

Figure 2

Blood Glucose
Insulin Delivery Rate
Glucagon Delivery Rate

Glucose (mg/dl)

Insulin (U/hr) & Glucagon (μg/min)

Study Time (minutes)
Figure 3

A

B