Exendin-(9-39) Effects on Glucose and Insulin in Children With Congenital Hyperinsulinism During Fasting and During a Meal and a Protein Challenge

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OBJECTIVE
The aim of this study was to assess whether exendin-(9-39) will increase fasting and postprandial plasma glucose and decrease the incidence of hypoglycemia in children with hyperinsulinism (HI).

RESEARCH DESIGN AND METHODS
This was an open-label, four-period crossover study. In periods 1 and 2, the effect of three different dosing regimens of exendin-(9-39) (group 1, 0.28 mg/kg; group 2, 0.44 mg/kg; group 3, 0.6 mg/kg) versus vehicle on fasting glucose was assessed in 16 children with HI. In periods 3 and 4, a subset of eight subjects received either vehicle or exendin-(9-39) (0.6 mg/kg) during a mixed-meal tolerance test (MMTT) and an oral protein tolerance test (OPTT).

RESULTS
Treatment group 2 showed 20% (P = 0.037) increase in the area under the curve (AUC) of fasting glucose. A significant increase in AUC of glucose was also observed during the MMTT and OPTT; treatment with exendin-(9-39) resulted in 28% (P = 0.001) and 30% (P = 0.01) increase in AUC of glucose, respectively. Fasting AUC of insulin decreased by 57% (P = 0.009) in group 3. In contrast, AUC of insulin was unchanged during the MMTT and almost twofold higher (P = 0.004) during the OPTT with exendin-(9-39) treatment. In comparison with vehicle, infusion of exendin-(9-39) resulted in significant reduction in likelihood of hypoglycemia in group 2, by 76% (P = 0.009), and in group 3, by 84% (P = 0.014). Administration of exendin-(9-39) during the OPTT resulted in 82% (P = 0.007) reduction in the likelihood of hypoglycemia.

CONCLUSIONS
These results support a therapeutic potential of exendin-(9-39) to prevent fasting and protein-induced hypoglycemia in children with HI.

Congenital hyperinsulinism (HI) is the most common cause of persistent hypoglycemia in infants and children. There are more than 11 known monogenic causes of HI, but the underlying genetic cause remains unknown in ~50% of the cases (1,2). Inactivating mutations in ABCC8 and KCNJ11, the genes encoding the two subunits...
of the β-cell K<sub>ATP</sub> channel, cause the most common and severe form of HI (known as K<sub>ATP</sub>HIL) (3). The phenotype of K<sub>ATP</sub>HIL is characterized by fasting and protein-induced hypoglycemia that is unresponsive to therapy with diazoxide, a K<sub>ATP</sub> channel activator (4). The likely cause for observed hypoglycemia after ingestion of protein is the glutamine-stimulated amplification of glucagon-like peptide 1 (GLP-1) receptor signaling on the β-cell (5).

GLP-1 is a hormone secreted from the intestinal L cells in response to ingested nutrients. In addition to exhibiting strong insulinotropic action, for which it was termed an incretin hormone, GLP-1 glucose-lowering action has been attributed to its ability to suppress glucagon secretion, endogenous glucose production (EGP), gastric emptying, and appetite and to enhance peripheral glucose disposal (6–8). We previously showed that a short-term intravenous infusion of exendin-(9–39), a GLP-1 receptor antagonist, significantly increased fasting glucose concentration in adolescents and adults with K<sub>ATP</sub>HIL (5). Furthermore, exendin-(9–39) inhibits amino acid–stimulated insulin secretion in pancreatic islets isolated from neonates with K<sub>ATP</sub>HIL (5), as well as in mouse islets lacking K<sub>ATP</sub> channels (9). These promising results suggest that the cardinal features of K<sub>ATP</sub>HIL can be reversed by inhibiting GLP-1 signaling. In this study, we enrolled younger children with HI and examined the effect of exendin-(9–39) not only during fasting but also in the postprandial state. We hypothesized that exendin-(9–39) will increase fasting and postprandial (mixed-meal and protein challenge) plasma glucose concentration and decrease the incidence of hypoglycemia (defined as plasma glucose <3.9 mmol/L [<70 mg/dL]) in children with HI.

**RESEARCH DESIGN AND METHODS**

**Subjects**

A total of 16 children (6 female and 10 male) ages 10 months–15 years with persistent hypoglycemia due to HI were included in the study. All but one had genetic confirmation of K<sub>ATP</sub>HIL; the subject without genetic confirmation had a phenotype consistent with K<sub>ATP</sub>HIL. Treatments for HI were withdrawn prior to the study; octreotide was stopped 48 h before the study, diazoxide was stopped 72 h before the study, and enteral dextrose was stopped before the fasting period started.

**Study Design**

This was an open-label, four-period crossover study to evaluate the effect of the GLP-1 receptor antagonist exendin-(9–39) on glucose metabolism in children with HI (trial reg. no. NCT00897676, ClinicalTrials.gov). The study was approved by the Human Subjects Committee of The Children’s Hospital of Philadelphia and the U.S. Food and Drug Administration. Written informed consent was obtained from all parents/guardians. Assent was obtained from the children when appropriate.

**Experimental Protocol**

The study was conducted in the inpatient unit of The Children’s Hospital of Philadelphia Center for Human Phenomic Science. All subjects were administered 5 ng exendin-(9–39) (0.05 μg/ml) intradermally as a test of immediate hypersensitivity prior to the study. Baseline chemistry profiles were obtained to evaluate liver and kidney function in all subjects, and a pregnancy test was performed in all postmenarchal females. Subjects participated in one or two experimental protocols as outlined here and depicted in Fig. 1.

**Study of Effects of Exendin-(9–39) During Fasting**

The effects of exendin-(9–39) during fasting were compared with those with vehicle (0.9% NaCl) infusion, assessed over a 2-day period (periods 1 and 2): subjects started fasting after a bedtime snack and ~12 h later received an intravenous infusion of vehicle (0.9% NaCl) for 1 h followed by either exendin-(9–39) or vehicle infusion for a total of six additional hours in random order on two successive days (Fig. 1). Subjects were assigned to three different treatment protocols of exendin-(9–39). The first group of subjects (treatment group 1, n = 6) was administered exendin-(9–39) at a rate of infusion of 300 pmol/kg/min (0.06 mg/kg/h) for 2 h, and then the rate of infusion was reduced to 100 pmol/kg/min (0.02 mg/kg/h) for 2 h and then returned back to 300 pmol/kg/min (0.06 mg/kg/h) for the final 2 h (total dose 0.28 mg/kg). The second group (treatment group 2, n = 7) received infusion of exendin-(9–39) at a rate of 300 pmol/kg/min (0.06 mg/kg/h) for 2 h, and then the infusion rate was increased to 500 pmol/kg/min (0.1 mg/kg/h) for another 2 h and, finally, the infusion rate was returned to the rate of 300 pmol/kg/min (0.06 mg/kg/h) for 2 h (total dose 0.44 mg/kg). The third group (treatment group 3, n = 3) received an infusion of exendin-(9–39) at a fixed rate of 500 pmol/kg/min (0.1 mg/kg/h) for 6 h (total dose 0.6 mg/kg). Blood samples were obtained from an indwelling catheter at different time points to measure plasma glucose, glucagon, total and active GLP-1, insulin, and C-peptide. For safety reasons, point-of-care (POC) glucose was monitored frequently (every 20–30 min), and an intravenous infusion of dextrose 10% was initiated if the plasma glucose decreased to <3.1 mmol/L (55 mg/dL).

**Study of Effects of Exendin-(9–39) During a Mixed-Meal Tolerance Test and an Oral Protein Tolerance Test**

The effects of exendin-(9–39) during a mixed-meal tolerance test (MMTT) and an oral protein tolerance test (OPTT) compared with vehicle infusion were assessed over a 2-day period (periods 3 and 4): subjects received either vehicle or exendin-(9–39) at a rate of 500 pmol/kg/min (0.1 mg/kg/h) intravenously for 6 h during an MMTT and an OPTT in random order on two successive days. After an overnight fast, an infusion of vehicle (0.9% NaCl) was run for 60 min (time –60 to 0) before the study infusion was started. At time zero the study infusions were started [vehicle or exendin-(9–39)] and subjects ate a solid breakfast meal consisting of 55% carbohydrate, 15% protein, and 30% fat that provided one-third of daily caloric requirement according to age and weight of the subject. Subjects consumed the same meal on both study days. At 3 h after the breakfast meal, subjects drank a protein shake (Resource Protein powder, 1 g/kg; Novartis). Plasma glucose, glucagon, total and active GLP-1, insulin, and C-peptide were measured at multiple time points. For safety reasons, POC glucose was monitored frequently (every 20–30 min), and an intravenous infusion of dextrose 10% was initiated if the plasma glucose decreased to <3.1 mmol/L (55 mg/dL).
Peptide
Exendin-(9-39) was synthesized by the American Peptide Company (Sunnyvale, CA) in accord with Current Good Manufacturing Practice guidelines. The peptide was purified to ≥97% with high-performance chromatography, and the sequence and mass were verified. The peptide was stored in a lyophilized form at −20°C. For administration, the peptide was diluted in 0.9% NaCl and added to 0.25% human serum albumin (final concentration 0.1 mg/mL). Aliquots were tested for sterility and pyrogenicity through the Investigational Drug Service at the University of Pennsylvania. The use of synthetic exendin-(9-39) is approved by the U.S. Food and Drug Administration (IND 76612).

Assays
Plasma glucose was measured with the Nova StatStrip point-of-care glucose monitor (Nova Biomedical, Waltham, MA), plasma insulin was measured with an

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Figure 1—Study protocol. Vertical arrows indicate when samples were taken. Horizontal lines indicate infusion of vehicle and exendin-(9-39) (Ex-9).
ELISA kit (cat. no. 08-10-1113-99; ALPCO Diagnostics, Salem, NH), plasma C-peptide was measured with a radioimmunoassay kit (cat. no. HCP-20K; Millipore, Linco Research, St. Charles, MO), plasma glucagon was measured with a radioimmunoassay kit (cat. no. GL-32K; Millipore, Linco Research), plasma active GLP-1 was measured in samples collected with dipeptidyl peptidase 4 (Millipore, Linco Research) inhibitor (10 μL/mL blood) to prevent proteolytic cleavage with use of a GLP-1 ELISA kit (cat. no. EGLP-35K; Millipore, Linco Research), and total GLP-1 was measured with a GLP-1 ELISA kit (cat. no. EZGLP1T-36K; Millipore, Linco Research).

Data Analysis

Mathematical Model of Whole-Body Insulin Kinetics

The proposed model of insulin kinetics depicted in Fig. 2 is described as follows by this set of differential equations,

$$\frac{dI}{dt} = -p_I I(t) + p_R R(t)$$  
$$\frac{dC_{p1}}{dt} = - (k_{12} + k_{10}) C_{p1}(t) + k_{21} C_{p2}(t) + R(t)$$  
$$\frac{dC_{p1}}{dt} \approx \frac{C_{p1}(t+h) - C_{p1}(t)}{h} = \frac{\Delta C_{p1}}{h}$$  
$$R(t) = \frac{\Delta C_{p1}}{h} + (k_{12} + k_{10}) C_{p1}(t) - k_{21} C_{p2}(t)$$

where in the one-compartment model of insulin (equation 1), first proposed by Watanabe and Bergman (10), $p_I$ represents the fractional whole-body plasma insulin clearance with units of 1/min. $l(t)$ is the plasma insulin concentration at a given time, $t$. Parameter $p_R$ represents the fraction of insulin that survives the first-pass hepatic extraction (FPHE), while $R(t)$ represents the equimolar secretion of insulin and C-peptide by the β-cell. Here the $R(t)$ is unknown. To reconstruct the temporal profile of endogenous insulin secretion, we use the two-compartment model of C-peptide kinetics (equation 2) (11), with assumed known fractional disappearance rates ($k_{12}$, $k_{20}$, and $k_{21}$). $C_{p1}(t)$ is the C-peptide concentration in the central compartment, and $C_{p2}(t)$ is the concentration of C-peptide in the remote compartment. To resolve equation 2, $\frac{dC_{p1}}{dt}$ is replaced by approximation with discrete forward difference, equation 3. To calculate the minute-to-minute forward differences in equation 3, we use linear interpolated profile of C-peptide concentration with a step, $h = 1$. Equation 4 is used to calculate the minute-to-minute profile of endogenous secretion of insulin and C-peptide by the β-cell.

Statistical Analysis

All analyses were conducted with Stata 16MP (StataCorp, State College TX), with two-sided tests of hypotheses and with a P value < 0.05 as the criterion for statistical significance. Tests of normal distribution were performed to determine the extent of skewness. For data with normal distribution, mean and SD are reported. Significantly skewed data are reported as medians and interquartile ranges. Frequency counts and percentages are used for categorical variables.

The area under the curve (AUC) for the plasma concentration time curve of glucose, insulin, C-peptide, active GLP-1, total GLP-1, and glucagon was calculated with use of the trapezoidal rule. The peak concentration (Cmax) and the time of Cmax (Tmax) was also calculated. Multilevel mixed-effects linear regression was used to identify any potential significant effects of the treatment on the outcomes of interest with adjustment for the order of administration of exendin-(9-39). Random effects were set on the level of the individual subjects. To permit for departure from normality of the residuals, we used a robust estimation of the variance. Post hoc pairwise analysis was conducted for the purpose of estimation of the marginal means and effects. For adjustment for multiple comparisons, Fisher protected least significant difference was used. All results are reported as marginal means ± SE or marginal means and differences with 95% CI unless otherwise specified.

The likelihood of hypoglycemia was assessed with a multivariable logistic regression model with fixed effects set on the level of treatment and adjustment for the order of administration of exendin-(9-39). Random effects were set on the level of the individual subjects. The likelihood was reported as odds ratios (ORs) with 95% CI.

RESULTS

Subject Characteristics

A total of 16 children (6 female and 10 male) ages 10 months–15 years with persistent hypoglycemia due to HI were included in the study; a subset of eight (three female and five male) completed all four periods (Table 1). Three of the 16 subjects were not receiving any pharmacologic therapy for HI at the time of enrollment, but all had evidence of persistent hypoglycemia. Eight subjects had undergone pancreatectomy previously but had persistent hypoglycemia requiring ongoing treatment.

Effects of Exendin-(9-39) During Fasting

With adjustment for order of administration, AUC of fasting plasma glucose...
was significantly higher in treatment group 2 in comparison with vehicle (1,517 ± 62 vs. 1,827 ± 131 mmol · min/L [27,313 ± 1,124 vs. 32,890 ± 2,362 mg · min/dL], respectively; *P* = 0.037) (Table 2 and Fig. 3A). In comparison with vehicle, infusion of exendin-(9-39) resulted in significant reduction in likelihood of fasting hypoglycemia in group 2, by 76% (OR 0.24 [95% CI 0.09–0.7], *P* = 0.009), and in group 3, by 84% (OR 0.16 [95% CI 0.04–0.69], *P* = 0.014).

AUC of plasma insulin was significantly decreased in treatment group 3 (11,265 ± 1,854 for vehicle vs. 4,834 ± 1,986 pmol · min/L [1,622 ± 267 vs. 696 ± 286 μU · min/mL], *P* = 0.009) (Table 2 and Fig. 3B). We observed significant decrease in AUC of C-peptide for treatment group 2 (83 ± 11 nmol · min/L [250 ± 34 ng · min/mL], *P* = 0.01) and group 3 (46 ± 15 nmol · min/L [140 ± 46 ng · min/mL], *P* < 0.001) in comparison with vehicle (93 ± 13 nmol · min/L [282 ± 40 ng · min/mL], Table 2 and Fig. 3C). There were no significant changes in AUC of glucagon with treatment (Fig. 3D). Furthermore, no changes in the AUC of total or active GLP-1 were observed (Table 2).

**Effects of Exendin-(9-39) During an MMTT and an OPTT**

Overall, in comparison with the AUC for vehicle, the AUC of plasma glucose for the MMTT was significantly elevated, by 28%, with the administration of exendin-(9-39) (1,459 ± 184 vs. 1,859 ± 194 mmol · min/L [26,259 ± 3,306 vs. 33,470 ± 3,498 mg · min/dL], *P* = 0.001) (Fig. 4A). AUC plasma glucose for the OPTT was significantly greater for exendin-(9-39) compared with vehicle (698 ± 73 vs. 1,030 ± 90 mmol · min/L [12,570 ± 1,318 vs. 18,540 ± 1,621 mg · min/dL], *P* = 0.010) (Fig. 5A). While the AUC of plasma insulin was unchanged during the MMTT (*P* = 0.827) (Table 3 and Fig. 4B), during OPTT the AUC of plasma insulin was increased with exendin-(9-39) treatment by almost twofold (*P* = 0.004) (Table 3 and Fig. 5B). However, the AUC of C-peptide (surrogate indicator of insulin secretion) was not significantly changed (*P* = 0.059) (Figs. 4C and 5C). This observation was confirmed with the model-estimated AUC insulin secretion rate, which was not significantly different during exendin-(9-39) compared with vehicle infusion for the MMTT or the OPTT (*P* > 0.05) (Table 3). AUC of glucagon was significantly elevated during both experimental protocols (MMTT and OPTT) in the exendin-(9-39) group in comparison with vehicle (MMTT AUC of glucagon, vehicle 12,345 ± 1,631 vs. exendin-(9-39) 14,031 ± 1,421 pg/mL · min, *P* < 0.001; OPTT AUC of glucagon, vehicle 15,758 ± 3,613 vs. exendin-(9-39) 21,251 ± 1,501 pg/mL · min, *P* = 0.025). In similar fashion, both total and active GLP-1
were elevated in the exendin-(9-39) group in comparison with vehicle for both tests (Table 3).

The model of whole-body insulin kinetics was well resolved for all subjects with both tests (MMTT and OPTT) with unique estimates for both insulin fractional clearance rate (FCR) and FPHE of insulin (Table 3) and standardized residuals randomly distributed around 0 and within 2 SD. FCR of insulin was not affected by exendin-(9-39) treatment during the MMTT \((P = 0.383)\). During the OPTT, treatment with exendin-(9-39) resulted in a significant \((P = 0.017)\) (Table 3), \(\sim 30\%\), reduction of FCR. FPHE of insulin estimated from the MMTT showed a significant \(28\%\) reduction during treatment with exendin-(9-39) \((P < 0.008)\) as calculated with the model of Dalla Man et al. (12).

Effect of Exendin-(9-39) on Protein-Induced Hypoglycemia
With adjustment for the time when hypoglycemia was observed during the OPTT, treatment with exendin-(9-39) resulted in a significant reduction \((P = 0.007)\) in the likelihood of observing hypoglycemia (plasma glucose < 3.9 mmol/L \(< 70 \text{ mg/dL}\)) in response to the protein challenge. In comparison with vehicle administration in the same subjects, treatment with exendin-(9-39) resulted in 82% reduction in the likelihood of hypoglycemia (OR 0.177 [95% CI 0.035–0.895]). With adjustment for time during the OPTT, no significant changes \((P = 0.075)\) between vehicle and treatment were observed in terms of likelihood of observing more severe hypoglycemia (plasma glucose < 3.3 mmol/L \(< 60 \text{ mg/dL}\)).

Safety of Exendin-(9-39)
Exendin-(9-39) was well tolerated by all subjects. There were no serious adverse events during the study. One subject experienced emesis during the infusion of exendin-(9-39) but did not require any intervention.

CONCLUSIONS
In this study, we examined the effect of exendin-(9-39) on fasting and protein-induced hypoglycemia, the cardinal phenotypic features of \(K_{\text{ATP}}\)HI. In the fasting state, treatment with a total dose of exendin-(9-39) of 0.44 mg/kg (group 2) resulted in significantly higher AUC of fasting plasma glucose concentration in comparison with vehicle. Importantly, frequency of fasting and protein-induced hypoglycemia was significantly reduced by exendin-(9-39).
We previously reported that in adolescents and adults with KATPHI, exendin-(9-39) increased fasting glucose and decreased insulin-to-glucose ratio (5). Furthermore, our previous studies in isolated K ATPHI human islets clearly demonstrate inhibition of amino acid–stimulated insulin secretion (5). These findings suggest that the mechanism of action for the observed effects on plasma glucose are mediated by the effects of exendin-(9-39) on the β-cell, however, the results of the current study suggest that there may be pancreatic and extrapancreatic effects of exendin-(9-39), all resulting in a beneficial glycemic response. While our current data indicate that in the fasting state the observed increase in AUC of plasma glucose was due to decreased insulin secretion secondary to exendin-(9-39) administration with no alterations in glucagon secretion, changes in postprandial glucose concentration during the MMTT were not associated with any changes in insulin secretion. Furthermore, the mechanisms resulting in elevated postprandial glucose concentration appear distinctly different between the two experimental protocols. Exendin-(9-39) administration during the MMTT caused insulin resistance that resulted in increased AUC of glucose and a possible increase in EGP due to reduced FPHE (Table 3). During the OPTT, exendin-(9-39) resulted in a significant, 30%, decrease in FCR (P = 0.017) (Table 3), which was responsible for the observed increased AUC of plasma insulin during the OPTT, with no change in insulin secretion (Table 3). However, AUC of plasma glucagon during the OPTT was significantly increased by exendin-(9-39), which may explain the reduction in protein-induced hypoglycemia.

It is important to note that the current study design was not optimal for a comprehensive assessment of the mechanisms of exendin-(9-39) action during a meal and protein challenge. Nevertheless, our observations allude to a possible mechanism of action in children with congenital HI. Of note, the choice of doses and dosing regimen in this study was limited by the lack of comprehensive toxicology and pharmacology data to support the use of higher doses; thus, it is possible that the dose of exendin-(9-39) has to be increased in order to observe inhibition of insulin secretion in the postprandial state. We previously reported that the half-life of exendin-(9-39) is 2.34 h in pediatric subjects and 1.81 h in adults (13); thus, it is expected that steady-state exendin-(9-39) concentrations were achieved during the 6-h infusions.

Due to sample size limitations in our study, the possibility still exists that the treatment may result in decreased insulin secretion. In HI, small changes in insulin secretion lead to large changes in plasma glucose concentrations; thus, to power a study that will detect such a small change, several-fold higher sample sizes may be required. Such an endeavor is a
Table 1—Subject characteristics

|                     | All four experiments | No OPTT or MMTT |
|---------------------|----------------------|-----------------|
| **n**               | 3 female, 5 male     | 3 female, 5 male|
| Age at enrollment, median (range) | 8.5 years (3–15 years) | 2.5 years (10 months–7 years) |
| **Genotype/phenotype** |                      |                 |
| ABCC8, dominant diazoxide responsive | 4                   | 1               |
| ABCC8, dominant diazoxide unresponsive | 2                   | 0               |
| ABCC8, biallelic recessive diazoxide unresponsive | 2                   | 4               |
| ABCC8, monoallelic recessive diazoxide unresponsive | 0                   | 1               |
| KCNJ11, dominant diazoxide responsive | 0                   | 1               |
| Unknown, partially diazoxide responsive | 0                   | 1               |
| **Pancreatectomy**  |                      |                 |
| No                  | 5                    | 3               |
| 55% resection       | 0                    | 1               |
| 98% resection       | 3                    | 4               |
| **HI management at enrollment** |                      |                 |
| Diazoxide           | 4                    | 1               |
| Octreotide          | 1                    | 0               |
| Enteral 20% dextrose | 0                   | 2               |
| Diazoxide + enteral 20% dextrose | 0                   | 2               |
| Octreotide + enteral 20% dextrose | 1                   | 2               |
| None                | 2                    | 1               |

Data are n unless otherwise indicated.

Table 2—Summary statistics of temporal profiles of glucose, insulin, C-peptide, glucagon, total GLP-1, and active GLP-1 for vehicle and exendin-(9-39) treatment groups

|                      | Vehicle | Treatment group 1 | Treatment group 2 | P       | Treatment group 3 | P       |
|----------------------|---------|-------------------|-------------------|---------|-------------------|---------|
| **Glucose**          |         |                   |                   |         |                   |         |
| AUC (mg/dL·min)      | 2,7312.94 ± 1,124.83 | 30,945.98 ± 2,011.99 | 32,889.67 ± 2,362.37 | *       | 31,238.10 ± 3,491.33 | **      |
| Cmax (mg/dL)         | 95.31 ± 7.00  | 104.65 ± 8.82     | 108.24 ± 8.45     |         | 105.00 ± 17.48    |         |
| Tcmax (min)          | 33.42 ± 19.44 | 74.12 ± 21.14     | 109.02 ± 37.81    |         | 195.79 ± 54.92    | **      |
| **Insulin**          |         |                   |                   |         |                   |         |
| AUC (μU/mL·min)      | 1,621.54 ± 266.78 | 1,491.97 ± 193.21 | 1,415.49 ± 193.21 | **      | 695.82 ± 285.74  | **      |
| Cmax (μU/mL)         | 5.89 ± 1.10   | 5.11 ± 1.06       | 5.08 ± 0.94       |         | 2.95 ± 1.02       | **      |
| Tcmax (min)          | 251.57 ± 37.16 | 270.69 ± 43.35    | 250.94 ± 40.85    |         | 291.40 ± 58.83   |         |
| **C-peptide**        |         |                   |                   |         |                   |         |
| AUC (ng/mL·min)      | 282.58 ± 39.36 | 270.75 ± 39.67    | 249.89 ± 34.41    | **      | 140.35 ± 45.87   | ***     |
| Cmax (ng/mL)         | 0.98 ± 0.15   | 0.94 ± 0.15       | 0.94 ± 0.14       |         | 0.58 ± 0.14      | ***     |
| Tcmax (min)          | 83.99 ± 32.45 | 71.90 ± 32.90     | 57.17 ± 45.78     |         | −5.12 ± 17.61    | *       |
| **Glucagon**         |         |                   |                   |         |                   |         |
| AUC (pg/mL·min)      | 22,875.82 ± 2,653.40 | 23,572.46 ± 2,904.13 | 23,929.40 ± 2,690.01 | **      | 22,191.67 ± 3,592.63 | **      |
| Cmax (pg/mL)         | 78.08 ± 7.93  | 76.38 ± 7.85      | 76.58 ± 7.76      |         | 74.92 ± 13.96    |         |
| Tcmax (min)          | 180.41 ± 28.11 | 249.87 ± 43.09    | 175.57 ± 47.02    |         | 291.75 ± 31.48   | **      |
| **Total GLP-1**      |         |                   |                   |         |                   |         |
| AUC (pmol/L·min)     | 4,904.45 ± 676.28 | 6,046.20 ± 2,165.59 | 5,588.14 ± 907.55 |         | 5,400.13 ± 1,053.32 | **      |
| Cmax (pmol/L)        | 18.85 ± 2.62  | 21.51 ± 5.83      | 21.69 ± 3.67      |         | 16.05 ± 4.31     |         |
| Tcmax (min)          | 166.44 ± 37.98 | 292.58 ± 36.71    | 304.11 ± 31.58    |         | 301.77 ± 23.49   | **      |
| **Active GLP-1**     |         |                   |                   |         |                   |         |
| AUC (pmol/L·min)     | 1,378.76 ± 260.63 | 1,274.50 ± 244.32 | 1,550.44 ± 340.52 |         | 1,469.20 ± 351.98 | **      |
| Cmax (pmol/L)        | 5.44 ± 1.00   | 3.81 ± 0.79       | 6.65 ± 1.73       |         | 4.78 ± 1.19      | **      |
| Tcmax (min)          | 192.08 ± 36.08 | 193.72 ± 56.31    | 262.71 ± 55.16    |         | 215.12 ± 29.18   | **      |

Data are statistical model–adjusted means ± SE. Treatment group 1, exendin-(9-39) infusion rate 300 pmol/kg/min for 2 h, then 100 pmol/kg/min for 2 h, and then 300 pmol/kg/min for 2 h; treatment group 2, exendin-(9-39) infusion rate 300 pmol/kg/min for 2 h, then 500 pmol/kg/min for 2 h, and then 300 pmol/kg/min for 2 h; and treatment group 3, exendin-(9-39) infusion rate 500 pmol/kg/min for 6 h. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
The liver itself extracts a large portion (>50%) of the insulin that is secreted into the portal vein. Meals rich in carbohydrates alter this proportion (17). It has been proposed that insulin in the liver directly suppresses EGP (18). That treatment with GLP-1 receptor agonists result in an increase in the fraction of insulin extracted by the liver has been shown (19). Thus, a smaller fraction of insulin survives the hepatic extraction and reaches the periphery. The reverse would be expected with GLP-1 receptor antagonism where GLP-1 receptor antagonism during the MMTT test result in decreased first-pass hepatic extraction of insulin and increased EGP. In this study, we show that in children with $K_{ATP,HI}$, GLP-1 receptor antagonism resulted in a 28% decrease in the hepatic insulin extraction during the MTTT. We assume that this increase in peripheral insulin, while relatively small, was sufficient to result in higher EGP and is responsible for the observed overall increase in AUC of glucose during the MMTT (Table 3).

However, it is also possible that the observed significant increase in plasma glucagon (Table 3) may have resulted in elevated EGP during the MTTT and the OPTT. We did not measure EGP in this study. Nevertheless, we hypothesize that the reduced hepatic extraction of insulin observed with the treatment combined with elevated glucagon levels may lead to increased EGP, as demonstrated by previous observations (2). As we and others previously reported (20,21), the GLP-1 response to a meal was significantly higher during exendin-(9-39) infusion.

Because these studies were performed only in children with HI, these observations cannot be generalized to healthy children. While it would have been interesting to replicate these studies in healthy children, such studies probably cannot be ethically justified.

In conclusion, here we show that treatment with exendin-(9-39) can successfully prevent fasting and protein-induced hypoglycemia in children with congenital HI. These findings add to the evidence previously published by us (5) supporting exendin-(9-39) as a potential treatment for hyperinsulinemic hypoglycemia. In addition, we provide evidence that beneficial effects of GLP-1 receptor antagonism on glucose in HI are mediated by several mechanisms—not only through inhibition of insulin secretion. Available medical treatments for HI are currently limited, insufficiently effective, and associated with significant side effects (22–24); by targeting the underlying pathophysiology, exendin-(9-39) offers potential therapeutic advantages over currently available therapies for HI, particularly for cases due to inactivating $K_{ATP}$ channel mutations that are not responsive to diazoxide. Furthermore, exendin-(9-39) has been well tolerated without significant treatment-related side effects in children with HI and also in adults with post–bariatric surgery hypoglycemia (25,26). Exendin-(9-39) has been granted breakthrough therapy designation for the

| Table 3—Summary statistics of temporal profiles of glucose, insulin, C-peptide, glucagon, total GLP-1, and active GLP-1 for vehicle and exendin-(9-39) during MMTT and OPTT |
|-----------------|-----------------|-----------------|-----------------|
|                 | **MMTT**        | **OPTT**        |                 |
| **Placebo**     | **Exendin-(9-39)** | **Placebo** | **Exendin-(9-39)** |
| **AUC glucose (mg kcal/min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 26,259          | 3,306           | 33,470          | 3,498           | **0.001**       | 12,570          | 1,318           | 18,540          | 1,621           | 0.01 |
| **AUC insulin (pmol/l - min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 25,811          | 6,507           | 25,227          | 5,597           | 0.827           | 14,584          | 4,141           | 27,797          | 6,139           | 0.004 |
| **AUC C-peptide (pmol/l - min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 171,367         | 22,443          | 179,653         | 29,360          | 0.534           | 139,776         | 24,075          | 183,620         | 17,283          | 0.059 |
| **AUC glucagon (pg/ml - min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 12,345          | 1,631           | 14,031          | 1,421           | **0.001**       | 15,757          | 3,613           | 21,251          | 1,501           | 0.025 |
| **AUC active GLP-1 (pmol/l - min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 1,153           | 179             | 2,362           | 493             | **0.001**       | 1,206           | 262             | 3,086           | 650             | **0.001** |
| **AUC total GLP-1 (pmol/l - min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 3,497           | 352             | 5,575           | 515             | **0.001**       | 4,611           | 973             | 7,538           | 1,060           | **0.001** |
| **AUC ISR (pmol/l - min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 11,815          | 1,436           | 12,389          | 1,773           | 0.562           | 12,135          | 1,802           | 11,329          | 1,373           | 0.553 |
| **FCR (1/min)** | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 0.219           | 0.086           | 0.264           | 0.072           | 0.383           | 0.309           | 0.071           | 0.225           | 0.051           | 0.017 |
| **FPHE (%)**    | Mean | SEM | Mean | SEM | **P** | Mean | SEM | Mean | SEM | **P** |
| 75              | 6               | 54              | 8               | **0.008**       | 48              | 9               | 54              | 8               | 0.566 |

Statistically significant findings are depicted in bold. ISR, insulin secretion rate.
treatment of HI (27), and future studies to evaluate efficacy and safety of multiple dose regimens are under development.

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Author Contributions. D.S. performed the modeling and statistical analysis and wrote and edited the manuscript. M.E.V. contributed to data preparation and analysis and edited the manuscript. S.G. researched data and reviewed the manuscript. D.D.D.L. designed the experiments, analyzed data, and edited the manuscript. D.D.D.L. 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.

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