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% ($P \leq 0.001$) 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

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of the β-cell K_{ATP} channel, cause the most common and severe form of HI (known as K_{ATP}HI) (3). The phenotype of K_{ATP}HI is characterized by fasting and protein-induced hypoglycemia that is unresponsive to therapy with diazoxide, a K_{ATP} 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_{ATP}HI (5). Furthermore, exendin-(9-39) inhibits amino acid–stimulated insulin secretion in pancreatic islets isolated from neonates with K_{ATP}HI (5), as well as in mouse islets lacking K_{ATP} channels (9). These promising results suggest that the cardinal features of K_{ATP}HI 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_{ATP}HI; the subject without genetic confirmation had a phenotype consistent with K_{ATP}HI. 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).

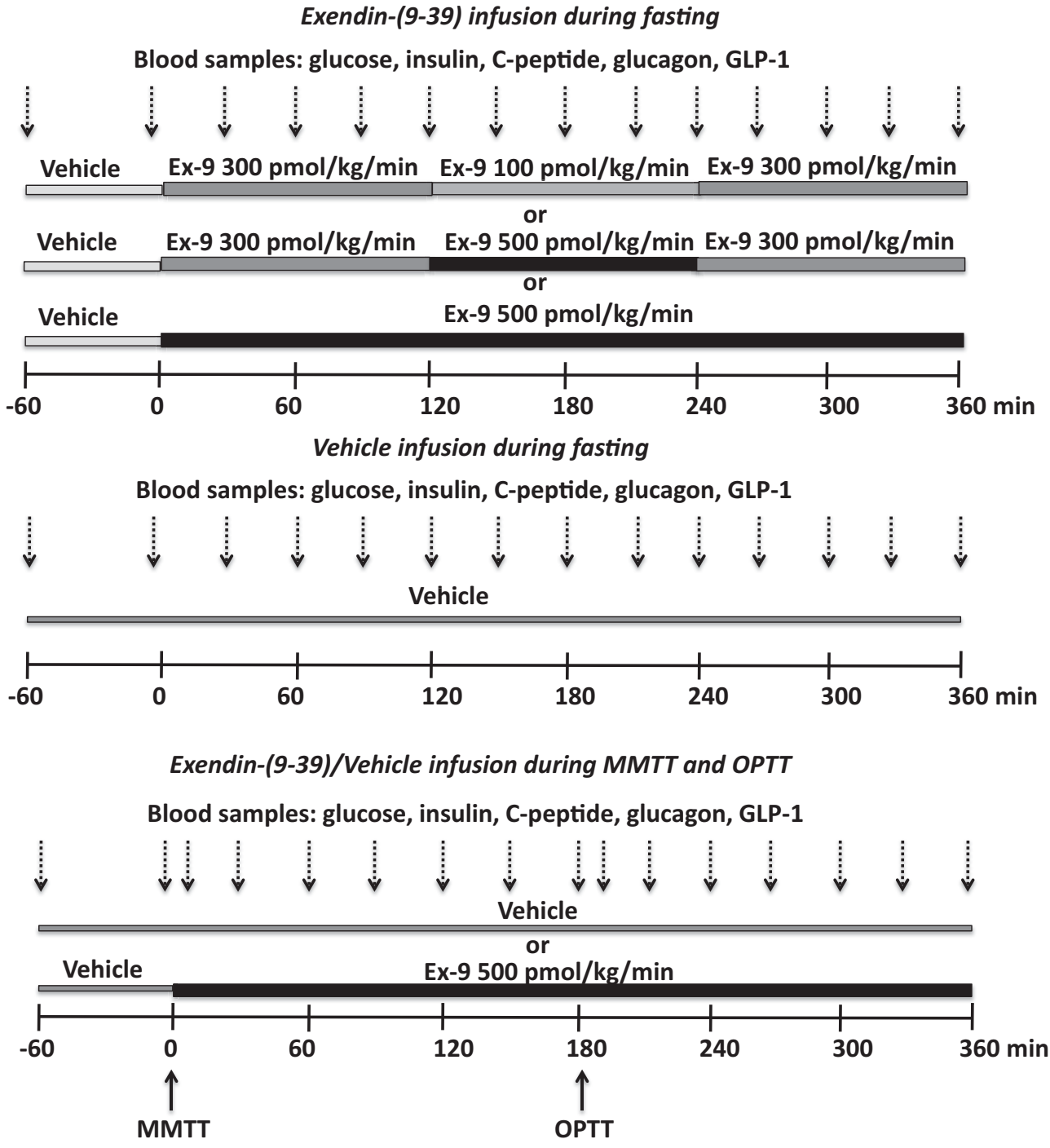

Figure 1—Study protocol. Vertical arrows indicate when samples were taken. Horizontal lines indicate infusion of vehicle and exendin-(9-39) (Ex-9).

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

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,

$$\frac{dI}{dt} = -p_I I(t) + p_{fp} R_a(t) \quad (1)$$

$$\frac{dC_{p1}}{dt} = -(k_{12} + k_{10}) C_{p1}(t) + k_{21} C_{p2}(t) + R_a(t) \quad (2)$$

$$\frac{dC_{p1}}{dt} \approx \frac{C_{p1}(t+h) - C_{p1}(t)}{h} = \frac{\Delta C_{p1}}{h} \quad (3)$$

$$R_a(t) = \frac{\Delta C_{p1}}{h} + (k_{12} + k_{10}) C_{p1}(t) - k_{21} C_{p2}(t) \quad (4)$$

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. *I(t)* is the plasma insulin concentration at a given time, *t*. Parameter p_{fp} represents the fraction of insulin that survives the first-pass hepatic extraction (FPHE), while $R_a(t)$ represents the equimolar secretion of insulin and C-peptide by the β-cell. Here the $R_a(t)$ 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_{10}, 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 (C_{max}) and the time of C_{max} (T_{Cmax}) 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.

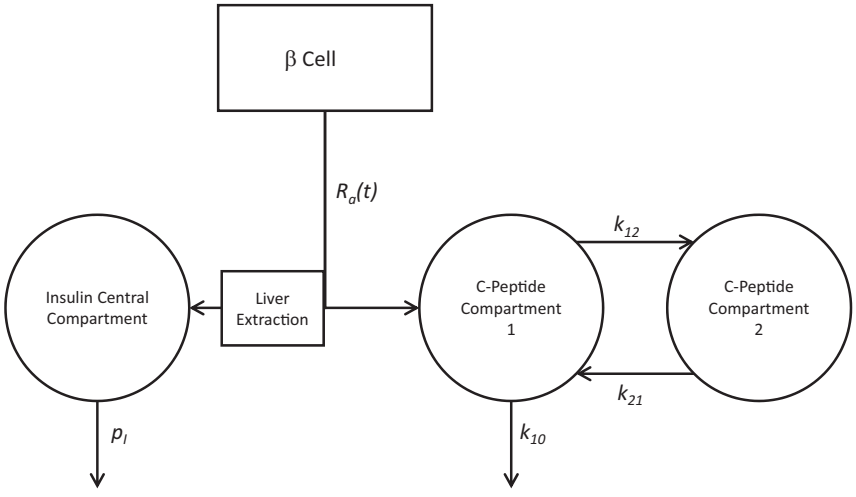

Figure 2—Graphical depiction of mathematical model of concurrent secretion and subsequent elimination of insulin and C-Peptide.

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

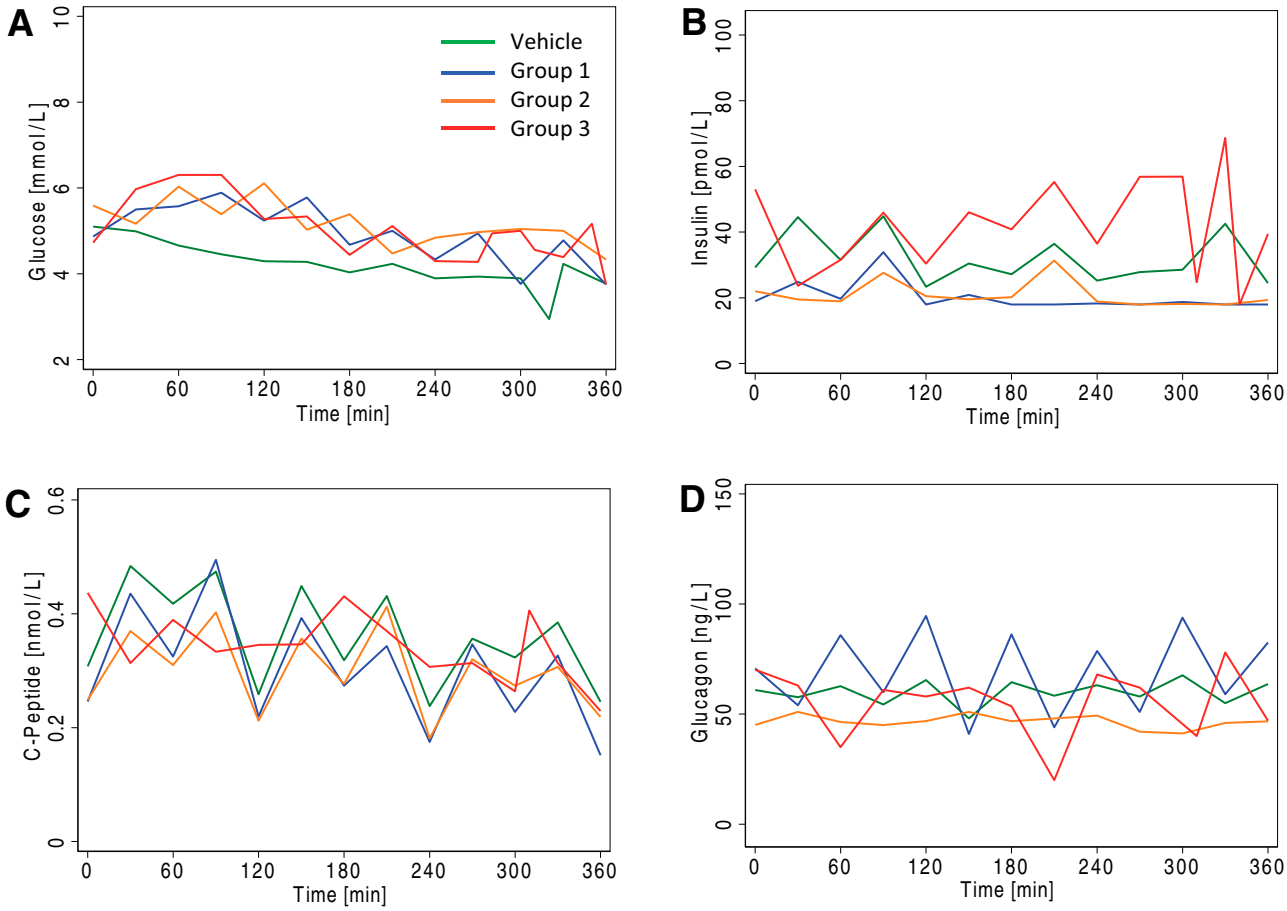

Figure 3—Average temporal fasting profile of plasma glucose (*A*), insulin (*B*), C-peptide (*C*), and glucagon (*D*) during infusion of vehicle; exendin-(9-39) with infusion rate 300 pmol/kg/min for 2 h, then 100 pmol/kg/min for 2 h, and then 300 pmol/kg/min for 2 h (group 1); exendin-(9-39) with infusion 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 (group 2); and exendin-(9-39) with infusion rate 500 pmol/kg/min for 6 h (group 3).

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. 3*A*). 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. 3*B*). 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. 3*C*). There were no significant changes in AUC of glucagon with treatment (Fig. 3*D*). 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], $P \leq 0.001$) (Fig. 4*A*). 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. 5*A*). While the AUC of plasma insulin was unchanged during the MMTT ($P = 0.827$) (Table 3 and Fig. 4*B*), during OPTT the AUC of plasma insulin was increased with exendin-(9-39) treatment by almost twofold ($P = 0.004$) (Table 3 and Fig. 5*B*). However, the AUC of C-peptide (surrogate indicator of insulin secretion) was not significantly changed ($P = 0.059$) (Figs. 4*C* and 5*C*). 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

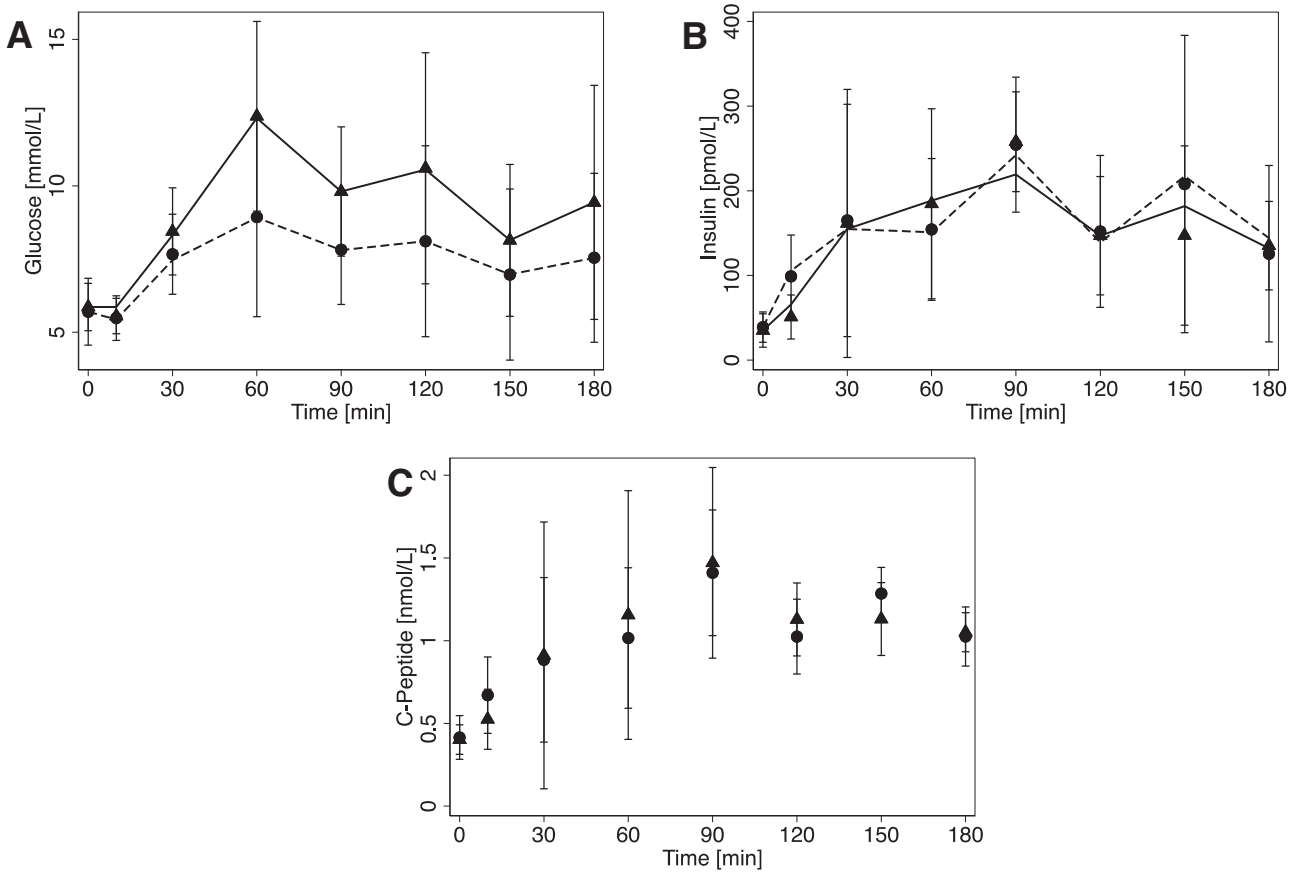

Figure 4—Average (with SE bars) temporal profiles of plasma glucose (*A*), insulin (*B*), and C-peptide (*C*) during MMTT for treatment with vehicle (●) and exendin-(9-39) at a rate of 500 pmol/kg/min (0.1 mg/kg/h) i.v. for 6 h (solid line, ▲). The lines in *A* and *B* represent the mathematical model fit for the vehicle (dashed line) and exendin-(9-39) (solid line) groups.

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), ∼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) (Table 3). Treatment with exendin-(9-39) did not result in a significantly further reduction or increase in FPHE during OPTT (Table 3). During the MMTT there was a significant reduction in insulin sensitivity (S_I), by 30%, for exendin-(9-39) versus vehicle (92.4 ± 38.2 vs. 64.6 ± 11.8 10^{-4} · pmol/L · min^{-1} [13.3 ± 5.5 vs. 9.3 ± 1.7 10^{-4} · μU/mL · min^{-1}], P < 0.015) 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 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 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_{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).

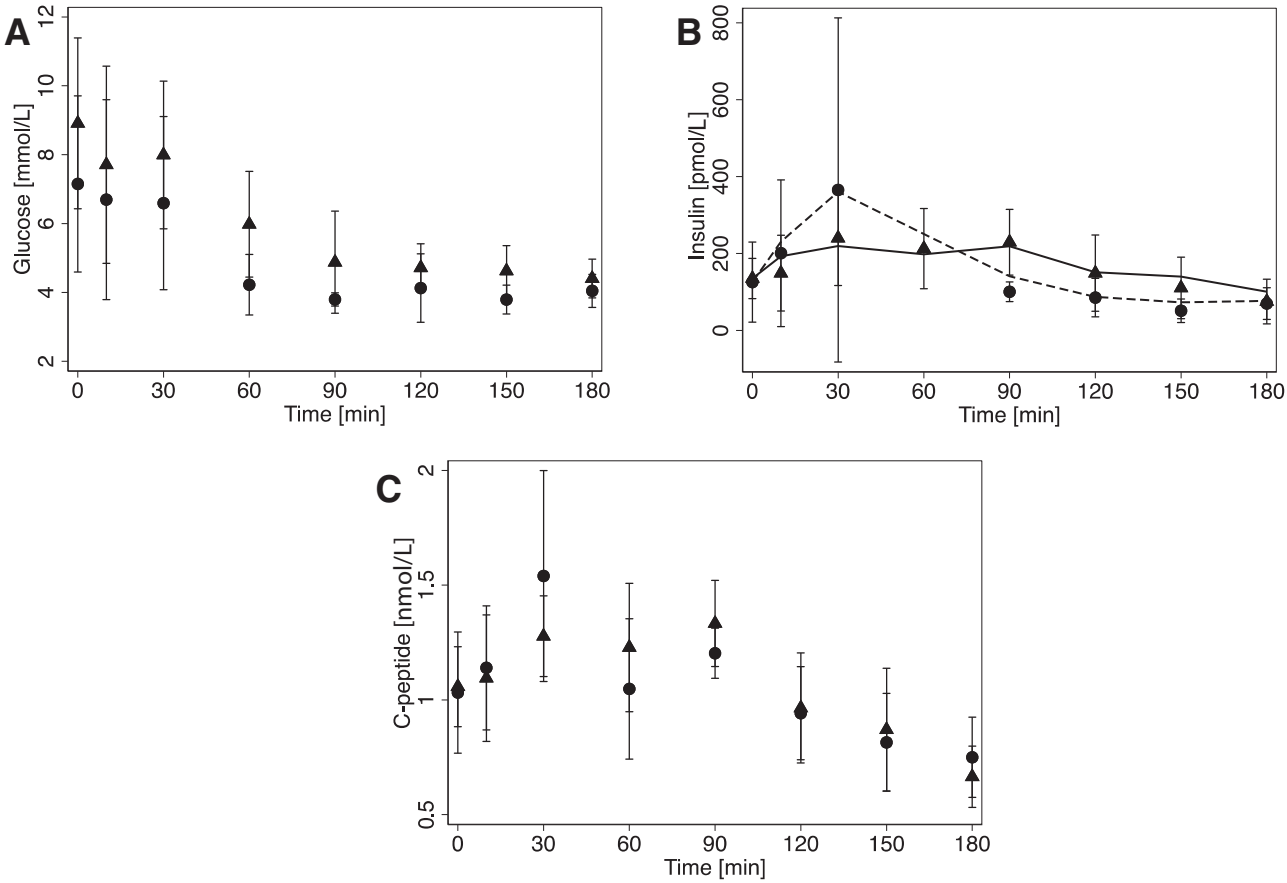

Figure 5—Average (with SE bars) temporal profiles of plasma glucose (*A*), insulin (*B*), and C-peptide (*C*) during OPTT for treatment with vehicle (dashed line, ●) and exendin-(9-39) at a rate of 500 pmol/kg/min (0.1 mg/kg/h) i.v. for 6 h (solid line, ▲). The lines in *B* represent the mathematical model fit for the vehicle (dashed line) and exendin-(9-39) (solid line) groups.

We previously reported that in adolescents and adults with K_{ATP}HI, exendin-(9-39) increased fasting glucose and decreased insulin-to-glucose ratio (5). Furthermore, our previous studies in isolated K_{ATP}HI 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	
C_{max} (mg/dL)	95.31 ± 7.00	104.65 ± 8.82	108.24 ± 8.45		105.00 ± 17.48	
T_{Cmax} (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	**
C_{max} (μU/mL)	5.89 ± 1.10	5.11 ± 1.06	5.08 ± 0.94		2.95 ± 1.02	**
T_{Cmax} (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	***
C_{max} (ng/mL)	0.98 ± 0.15	0.94 ± 0.15	0.94 ± 0.14		0.58 ± 0.14	***
T_{Cmax} (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	
C_{max} (pg/mL)	78.08 ± 7.93	76.38 ± 7.85	76.58 ± 7.76		74.92 ± 13.96	
T_{Cmax} (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 ± 1,654.59	5,558.14 ± 907.55		5,400.13 ± 1,053.32	
C_{max} (pmol/L)	18.85 ± 2.62	21.51 ± 5.83	21.69 ± 3.67		16.05 ± 4.31	
T_{Cmax} (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	
C_{max} (pmol/L)	5.44 ± 1.00	3.81 ± 0.79	6.65 ± 1.73		4.78 ± 1.19	
T_{Cmax} (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. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

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)		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	Mean	SEM	*P*
AUC glucose (mg · dL/min)	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)	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)	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)	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)	1,153	179	2,362	493	**≤0.001**	1,206	262	3,086	650	**≤0.001**
AUC total GLP-1 (pmol/L · min)	3,497	352	5,575	515	**≤0.001**	4,611	973	7,538	1,060	**≤0.001**
AUC ISR (pmol/L · min)	11,815	1,436	12,389	1,773	0.562	12,135	1,802	11,329	1,373	0.553
FCR (1/min)	0.219	0.086	0.264	0.072	0.383	0.309	0.071	0.225	0.051	**0.017**
FPHE (%)	75	6	54	8	**0.008**	48	9	54	8	0.566

Statistically significant findings are depicted in bold. ISR, insulin secretion rate.

significant challenge given the low prevalence of HI.

Based on previous data it has been hypothesized that GLP-1 may exhibit beneficial effects by promoting the peripheral disposal of glucose (8). There are two possible mechanism through which GLP-1 may achieve this function. First, it has been shown that insulin exhibits a strong vasodilation function that facilitates the delivery of oxygen, nutrients, and insulin to myocytes (14). It has also been shown that GLP-1 augments this function (15). Hence, it is possible that GLP-1 receptor antagonism leads to diminished delivery of nutrients in the periphery. Second and final, it has been shown that GLP-1 agonists can augment insulin action at the cellular level by increasing insulin-stimulated cell-surface expression of GLUT4 (16). Therefore, it is expected that GLP-1 receptor antagonism will diminish the translocation of GLUT4. Our observation of 30% decrease in insulin sensitivity during the MMTT is consistent with the above proposed mechanisms of extrapancreatic action of GLP-1 receptor antagonism. Furthermore, the observed decrease in FCR during the OPTT is also consistent with the peripheral role of GLP-1 receptor antagonism in insulin's whole-body kinetics. Unfortunately, there are no current indices of insulin sensitivity that can quantify insulin sensitivity during an OPTT. Future studies will have to be designed to assess the cellular effect of exendin-(9-39) during an OPTT.

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 will 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 MMTT 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

treatment of HI (27), and future studies to evaluate efficacy and safety of multiple dose regimens are under development.

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References

1. Snider KE, Becker S, Boyajian L, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. J Clin Endocrinol Metab 2013;98:E355–E363
2. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. J Clin Endocrinol Metab 2016;101:815–826
3. Vajravelu ME, De León DD. Genetic characteristics of patients with congenital hyperinsulinism. Curr Opin Pediatr 2018;30:568–575
4. Fourtner SH, Stanley CA, Kelly A. Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by K(ATP) channel mutations. J Pediatr 2006;149:47–52
5. Calabria AC, Li C, Gallagher PR, Stanley CA, De León DD. GLP-1 receptor antagonist exendin-(9-39) elevates fasting blood glucose levels in congenital hyperinsulinism owing to inactivating mutations in the ATP-sensitive K+ channel. Diabetes 2012;61:2585–2591
6. Nauck MA, Holst JJ, Willms B, Schmiegel W. Glucagon-like peptide 1 (GLP-1) as a new therapeutic approach for type 2-diabetes. Exp Clin Endocrinol Diabetes 1997;105:187–195
7. Nauck MA, Niedereichholz U, Ettler R, et al. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 1997;273:E981–E988
8. Nauck MA, Meier JJ. Incretin hormones: their role in health and disease. Diabetes Obes Metab 2018;20(Suppl. 1):5–21
9. De León DD, Li C, Delson MI, Matschinsky FM, Stanley CA, Stoffers DA. Exendin-(9-39) corrects fasting hypoglycemia in SUR-1-/- mice by lowering cAMP in pancreatic beta-cells and inhibiting insulin secretion. J Biol Chem 2008;283:25786–25793
10. Watanabe RM, Bergman RN. Accurate measurement of endogenous insulin secretion does not require separate assessment of C-peptide kinetics. Diabetes 2000;49:373–382
11. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368–377
12. Dalla Man C, Caumo A, Cobelli C. The oral glucose minimal model: estimation of insulin sensitivity from a meal test. IEEE Trans Biomed Eng 2002;49:419–429
13. Ng CM, Tang F, Seeholzer SH, Zou Y, De León DD. Population pharmacokinetics of exendin-(9-39) and clinical dose selection in patients with congenital hyperinsulinism. Br J Clin Pharmacol 2018;84:520–532
14. Bergman RN, Yang YJ, Hope ID, Ader M. The role of the transcapillary insulin transport in the efficiency of insulin action: studies with glucose clamps and the minimal model. Horm Metab Res Suppl 1990;24:49–56
15. Chai W, Fu Z, Aylor KW, Barrett EJ, Liu Z. Liraglutide prevents microvascular insulin resistance and preserves muscle capillary density in high-fat diet-fed rats. Am J Physiol Endocrinol Metab 2016;311:E640–E648
16. Li Z, Zhu Y, Li C, et al. Liraglutide ameliorates palmitate-induced insulin resistance through inhibiting the IRS-1 serine phosphorylation in mouse skeletal muscle cells. J Endocrinol Invest 2018;41:1097–1102
17. Campioni M, Toffolo G, Basu R, Rizza RA, Cobelli C. Minimal model assessment of hepatic insulin extraction during an oral test from standard insulin kinetic parameters. Am J Physiol Endocrinol Metab 2009;297:E941–E948
18. Edgerton DS, Kraft G, Smith M, et al. Insulin's direct hepatic effect explains the inhibition of glucose production caused by insulin secretion. JCI Insight 2017;2:e91863
19. Zheng D, Ionut V, Mooradian V, Stefanovski D, Bergman RN. Exenatide sensitizes insulin-mediated whole-body glucose disposal and promotes uptake of exogenous glucose by the liver. Diabetes 2009;58:352–359
20. Calabria AC, Charles L, Givler S, De León DD. Postprandial hypoglycemia in children after gastric surgery: clinical characterization and pathophysiology. Horm Res Paediatr 2016;85:140–146
21. Salehi M, Prigeon RL, D'Alessio DA. Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans. Diabetes 2011;60:2308–2314
22. Welters A, Lerch C, Kummer S, et al. Long-term medical treatment in congenital hyperinsulinism: a descriptive analysis in a large cohort of patients from different clinical centers. Orphanet J Rare Dis 2015;10:150
23. Herrera A, Vajravelu ME, Givler S, et al. Prevalence of adverse events in children with congenital hyperinsulinism treated with diazoxide. J Clin Endocrinol Metab 2018;103:4365–4372
24. Thornton P, Truong L, Reynolds C, Hamby T, Nedrelow J. Rate of serious adverse events associated with diazoxide treatment of patients with hyperinsulinism. Horm Res Paediatr 2019;91:25–32
25. Craig CM, Lawler HM, Lee CJE, et al. PREVENT: a randomized, placebo-controlled crossover trial of avexitide for treatment of postbariatric hypoglycemia. J Clin Endocrinol Metab 2021;106:e3235–e3248
26. Tan M, Lamendola C, Luong R, McLaughlin T, Craig C. Safety, efficacy and pharmacokinetics of repeat subcutaneous dosing of avexitide (exendin 9-39) for treatment of post-bariatric hypoglycaemia. Diabetes Obes Metab 2020;22:1406–1416
27. Eiger gets breakthrough therapy designation for congenital hyperinsulinism treatment, 2021. Accessed 31 December 2021. Available from https://www.fdanews.com/articles/203878-eiger-gets-breakthrough-therapy-designation-for-congenital-hyperinsulinism-treatment?v=preview