

JPET #69765

## **Exendin-4 Pharmacodynamics: Insights From the Hyperglycemic Clamp Technique**

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JPET #69765

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**Abbreviations:** GLP-1, glucagon-like peptide-1; MAP, Maximum *a Posteriori*; PK, pharmacokinetics; PD, pharmacodynamics.

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JPET #69765

## ABSTRACT

The purpose of this study is to ascertain the pharmacodynamic properties of exendin-4, a glucose-dependent insulinotropic agent, from plasma glucose and insulin concentration-time profiles following a 60-minute intravenous infusion in healthy and type 2 diabetic subjects. Plasma glucose and insulin concentrations were obtained from a previous clinical study, whereby a hyperglycemic clamp was established and maintained in healthy (n=7) and type 2 diabetic (n=7) volunteers (plasma glucose raised 5.4mmol/L above fasting level). Exendin-4 was infused (0.15pmol/kg/min) during the second hour of a 5-hour clamp. A physiological pharmacodynamic model was developed and fitted to individual glucose and insulin responses simultaneously. As drug concentrations were unavailable, hypothetical pharmacokinetic driving functions were approximated during the modeling process and used to enhance a proportionality constant relating elevated glucose and the rate of second-phase insulin release. Exendin-4 infusions produced substantial insulin release in both subject populations that required higher glucose infusion rates to maintain stable hyperglycemia. Observed plasma glucose-insulin profiles were well characterized by the final pharmacodynamic model. Apparent exendin-4 elimination rate constants for healthy and diabetic subjects were similar ( $0.0386 \pm 0.0192$  and  $0.0460 \pm 0.0145 \text{ min}^{-1}$ ). Capacity and sensitivity parameters of drug effect were 2-fold lower in diabetic subjects, but mean differences were not statistically significant. Simulations confirm that diabetic subjects exhibit a reduced capacity to enhance second-phase insulin release in response to exendin-4 as compared to healthy subjects. Type 2 diabetic subjects demonstrate a significant response to exendin-4, but to a lesser extent than nondiabetic subjects, despite comparable measures of apparent drug exposure and efficacy.

## JPET #69765

Pharmacological agents that directly modulate the secretion of insulin (insulinotropics) represent an important class of drugs for the treatment of diabetes mellitus (Doyle and Egan, 2003). Glucagon-like peptide-1 (GLP-1) is a potent glucose-dependent insulinotropic agent that also elicits multiple effects in  $\beta$ -cells that may provide further therapeutic benefits to diabetic patients including increase in  $\beta$ -cell mass (differentiation and neogenesis) and the number of cells secreting insulin, and upregulation of genes involved in insulin regulation (Doyle and Egan, 2001). However, the development of GLP-1 as a therapeutic drug is seriously limited by a short pharmacokinetic and biological half-life (Orskov et al., 1993), primarily resulting from in vivo hydrolysis by the enzyme dipeptidyl peptidase IV (Kieffer et al., 1995). In contrast, exendin-4 is a 39 amino acid peptide GLP-1 receptor agonist, produced in the saliva of the Gila monster lizard (*Heloderma suspectum*), that exhibits greater potency and a longer duration of action than GLP-1 (Goke et al., 1993; Thorens et al., 1993; Doyle and Egan, 2001). It contains amino acid substitutions so that it is not a substrate for dipeptidyl peptidase IV and a 9-amino acid C-terminal addition, which stabilizes the molecule on the GLP-1 receptor (Doyle et al., 2003). Exendin-4 has been shown to produce significant glucose-dependent insulinotropic effects in healthy and type 2 diabetic subjects (Edwards et al., 2001; Egan et al., 2002).

To date the in vivo pharmacological effects of exendin-4 have been evaluated either qualitatively (Egan et al., 2002) or by comparing empirical measures such as the area under the plasma glucose and insulin concentration-time curves (Edwards et al., 2001). On the contrary, mechanism-based pharmacodynamic (PD) models can be used to characterize the temporal and causal relationships between plasma drug concentrations (pharmacokinetics, PK) and biological responses (Mager et al., 2003). Early physiological models provided key quantitative insights into the control of insulin on glucose metabolism (Insel et al., 1975). Most contemporary

## JPET #69765

mathematical models of the glucose-insulin system rely on the so-called minimal model (Bergman et al., 1979) that is often applied in a piece-wise manner by fixing glucose and modeling insulin concentrations and/or vice-versa (Toffolo et al., 1980; Pacini et al., 1982). This integrative physiological approach has proven highly useful for over 20 years in the analysis of experimental data and, more importantly, for understanding the pathogenesis, clinical course, and treatment of diabetes (Bergman, 1989; Bergman, 2002). Recently, the minimal model was adapted to simultaneously evaluate the effects of NN2211, another GLP-1 derivative, on glucose and insulin concentrations over time during an intravenous (IV) glucose tolerance test in healthy volunteers (Agero and Vicini, 2003). This represents a more desirable case, where both sets of data are modeled simultaneously in a unified model that continues to capture the major features of this dynamical system. Similar modifications have also been used to model the PK/PD properties of insulin aspart and human insulin in healthy volunteers under euglycemic clamp conditions (Osterberg et al., 2003). In this study, the effects of exendin-4 on glucose-insulin homeostasis under hyperglycemic clamp conditions in healthy and type 2 diabetic subjects were quantified via a proposed mechanistic PD model.

## Methods

We obtained plasma glucose and insulin concentrations and glucose infusion rates from a recent clinical study (Egan et al., 2002). The previous study was approved by the Johns Hopkins Bayview Medical Center Institutional Review Board (with investigator initiated IND obtained from the FDA), and this secondary data analysis was approved by the MedStar Research Institute Institutional Review Board with a waiver from further informed consent.

**Database.** Details of the experimental design are fully described in the original study (Egan et al., 2002). Briefly, seven nondiabetic subjects (3 males, 4 females; 5 Caucasians, 2 African Americans) and seven non-insulin-treated type 2 diabetic subjects (5 males, 2 females; 2 Caucasians, 4 African Americans, 1 Hispanic) were enrolled. Age ranges were 24-56 years and 45-74 years, and body mass index ranges were 20.2-36.4 kg·m<sup>2</sup> and 32.7-46.8 kg·m<sup>2</sup> for healthy and diabetic subjects, respectively. Four of the seven diabetic volunteers were taking oral sulfonylureas, which were voluntarily withheld for 3 days prior to testing. After an overnight fast and determining a stable fasting state, a hyperglycemic clamp was initiated (Elahi, 1996), whereby plasma glucose levels were raised and maintained at 5.4 mmol/L above fasting levels for 5 hours. These elevated levels were held stable for the duration of the clamp by a variable glucose infusion rate, determined from algorithms based on the prevailing plasma glucose, which was measured at 5 minute intervals at the bedside (for a complete overview of clamp methodology see Elahi, 1996). Exendin-4 (AC2993; Amylin Pharmaceuticals, Inc., San Diego, CA) was diluted in 50 mL of normal saline containing 2 mL of each subject's blood and infused for 60 minutes during the second hour of the 5 hour clamp. The drug infusion rate was changed at 2 minute intervals from 0.59 to 0.25, 0.23, 0.22, and 0.20, and was held constant from 10 minutes to the end of the hour at 0.15 pmol/kg/min. Once the clamp was initiated, plasma

## JPET #69765

glucose and insulin concentrations were measured every 2 minutes for the first 10 minutes, and then every 5 minutes (glucose) or 10 minutes (insulin) until 30 minutes after the termination of the hyperglycemic clamp. Blood samples (3, 0.5, and 10 mL for 2, 5, and 10 minute samples respectively) were collected with heparinized syringes and processed as previously described (Elahi et al., 1993). Plasma glucose was measured immediately at the bedside using a Beckman Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, CA). The remaining blood was placed in pre-chilled test tubes containing aprotinin (400,000 IU/mL) and EDTA (1.5 mg/mL) and centrifuged at 4°C. Samples were stored at -70°C until analysis, where plasma insulin concentrations were measured by radioimmunoassay (Linco Research, Inc., St. Charles, MO) using the Human Insulin-specific RIA kit (Cat No. HI-14K). Glucose infusion rates were recorded (mg/kg/min) at the same intervals as the plasma glucose measurements.

**PD Model.** The proposed PD model is based on modifications by Agerso and Vicini to the minimal model paradigm (Agerso and Vicini, 2003) and a schematic is shown in Figure 1. Single compartments are shown for glucose ( $G$ ) and insulin ( $I$ ) concentrations, along with a separate remote insulin compartment ( $X$ ), which acts as an "effect" compartment. This system can be defined by the following series of differential equations:

$$dG/dt = R(t)/V - (X + p_1) \cdot G + p_1 \cdot G_0 \quad (1)$$

$$dX/dt = p_3 \cdot (I - I_0) - p_2 \cdot X \quad (2)$$

$$dI/dt = IR_1 + IR_2 + n \cdot (I_0 - I) \quad (3)$$

where  $R(t)$  is the variable glucose infusion rate,  $V$  is the glucose volume of distribution,  $p_1$  is the first-order insulin-independent loss rate of glucose,  $p_2$  is the first-order remote insulin removal rate constant,  $p_3$  represents insulin action or the product of insulin sensitivity and  $p_2$ ,  $n$  is the first-order insulin elimination rate constant, and  $IR_1$  and  $IR_2$  are the first and second phases of

JPET #69765

insulin release in response to elevated glucose concentrations. The initial conditions of equations 1 ( $G_0$ ) and 3 ( $I_0$ ) were fixed to mean values of 4 measurements made at 10 minute intervals just prior to initiating the hyperglycemic clamp, and the initial condition of equation 2 is zero.

Plasma glucose concentrations were modeled in units of mg/dL, whereas insulin concentrations were  $\mu\text{U/mL}$  and converted to pmol/L in the output equation ( $1 \mu\text{U/mL} = 6.0 \text{ pmol/L}$ ). The first-phase insulin release ( $IR_1$ ) was described by an empirical Gaussian function:

$$IR_1 = \frac{I_{rel}}{T_{dur} \cdot \sqrt{2\pi}} \cdot e^{-\frac{(t-T_{sec})^2}{2T_{dur}^2}} \quad (4)$$

where  $I_{rel}$  is the function amplitude,  $T_{dur}$  and  $T_{sec}$  are the duration and time of maximal insulin release, and  $t$  is time. The second-phase insulin release ( $IR_2$ ) is given by the following equation:

$$IR_2 = \left( \gamma + \frac{E_{max} \cdot A}{EA_{50} + A} \right) \cdot t \cdot (G - h) \quad (5)$$

where  $\gamma$  is a proportionality constant between elevated glucose and the rate of  $IR_2$ , and  $h$  is the threshold glucose concentration ( $IR_2 = 0$  for  $G < h$ ). As plasma exendin-4 concentrations were unavailable, a hypothetical PK function was defined for the amount of drug ( $A$ ) present at the biophase as a function of time (Gabrielsson et al., 2000):

$$dA/dt = K_0(t) - k_e \cdot A \quad (6)$$

where  $K_0(t)$  is the primed zero-order exendin-4 infusion rate and  $k_e$  is the first-order exendin-4 elimination rate constant. The initial condition of Equation 6 is obviously zero. The PK driving function is used to enhance  $\gamma$  with a standard  $E_{max}$  equation (Eq. 5), where  $E_{max}$  is the maximum effect and  $EA_{50}$  is the amount of exendin-4 producing 50% of  $E_{max}$ .

**Data and Statistical Analysis.** Areas under the glucose infusion rate- and plasma insulin concentration-time curves from time zero to 60 minutes ( $\text{AUC}_{0-60}$ ) were calculated using the

## JPET #69765

linear trapezoidal method. For insulin profiles, net AUC values were calculated according to,  $AUC_{net} = AUC_{0-60} - AUC_{baseline}$ , where  $AUC_{baseline} = I_0 \cdot 60$  min. Comparisons of AUCs between groups were made by the Mann-Whitney  $U$ -test conducted with GraphPad InStat (v3.0 for Win 95/NT, GraphPad Software, Inc., San Diego CA).

Individual subject data sets of plasma glucose and insulin concentration-time profiles were modeled simultaneously (Eqs. 1-6), and glucose infusion rates were entered as known values ( $R(t)$  in Eq. 1). Owing to the complexity of the model, parameters were estimated using Maximum *a Posteriori* (MAP) Bayesian estimation as implemented in the ADAPT II computer program (D'Argenio and Schumitzky, 1997). Where available, prior mean values were obtained from the literature and specified with empirical prior standard deviations (20-30% of mean values) and assuming log-normal distributions. No prior information was entered for  $I_{rel}$ ,  $E_{max}$ ,  $EA_{50}$  and  $k_e$  (i.e. non-informative priors). A standard variance model was specified:

$$\sigma_i^2(t_j) = (\sigma_i \cdot y_i(t_j))^2 \quad (7)$$

where separate variance model parameters ( $\sigma_i$ ) were used for glucose and insulin, and  $y_i(t_j)$  represents model predicted values. Goodness-of-fit and model selection was assessed using the generalized information criterion for MAP estimation (D'Argenio and Schumitzky, 1997), correlation coefficients, residual distributions, and visual inspection.

Final estimated parameters were reported as mean (CV%) for healthy and diabetic subjects. Unpaired Student t-tests with Welch correction were performed to assess differences between mean values using GraphPad InStat.

## Results

A square wave of hyperglycemia was achieved in both diabetic subjects and nondiabetic volunteers (Fig. 2, top panels). The mean time-zero or fasting glucose concentration was significantly higher in the diabetic group as compared to healthy subjects ( $G_0 = 193 \pm 68.3$  vs.  $99.3 \pm 5.5$  mg/dL;  $p < 0.01$ ), whereas fasting insulin was higher but the difference not statistically significant ( $I_0 = 18.0 \pm 7.9$  vs.  $12.6 \pm 6.9$   $\mu$ U/mL). During the first hour of the hyperglycemic clamp, there was an initial release of insulin (first-phase insulin release), which was less or absent for subjects in the diabetic group, followed by a linear increase in insulin concentrations (second-phase insulin release) over time (Fig. 2, bottom panels). The  $AUC_{0-60}$  values for the glucose infusion rates were not statistically significant ( $299 \pm 24$  vs.  $285 \pm 14$  mg/kg for healthy and diabetic subjects; mean  $\pm$  s.e.), whereas  $AUC_{0-60}$  values for insulin profiles were significantly greater in healthy subjects ( $15,800 \pm 8,100$  vs.  $2,570 \pm 940$  pmol·min/L; mean  $\pm$  s.e.;  $p = 0.007$ ). As would be expected, these AUC comparisons reflect a decreased capacity for glucose-dependent insulin release in diabetic subjects. A rapid and significant increase in insulin concentrations was observed in both groups upon the initiation of the exendin-4 infusion, and insulin concentrations remained above fasting levels for the duration of the hyperglycemic clamp (Fig. 2, bottom panels).

Individual sets of plasma glucose and insulin concentration-time profiles were modeled simultaneously using the proposed PD model (Fig. 1), and representative plots are shown in Figure 3. Although residual distributions appeared smaller for diabetic subjects, the data were well characterized overall with good agreement between model predicted and observed concentrations for both populations (Fig. 4). The mean estimated model parameters are listed in Table 1. Significant differences were found between the two groups for  $p_3$ ,  $\gamma$ ,  $h$ , and the two

## JPET #69765

variance model parameters. There was also a trend for lower values in the diabetic group for  $I_{rel}$ ,  $p_2$ ,  $E_{max}$ , and  $EA_{50}$ ; however, the differences were not statistically significant.

The pharmacological properties of exendin-4 were directly evaluated through PK/PD simulations of effective drug exposure ( $A$  in Eq. 6) and effective  $\gamma$  values ( $\gamma_{eff}$ ), defined as:

$$\gamma_{eff} = \gamma + E_{max} \cdot A / (EA_{50} + A) \quad (8)$$

Simulations over time were conducted using mean parameter estimates for  $k_e$ ,  $\gamma$ ,  $E_{max}$ , and  $EA_{50}$  (Table 1). The hypothetical PK profiles shown in Figure 5 (top panel) indicate that the PK properties of exendin-4 are expected to be similar between nondiabetic and diabetic subjects. Although apparent effective drug exposure was approximated to be similar between groups, the lower  $E_{max}$  term for diabetic volunteers, coupled with a lower baseline  $\gamma$  value, reflects a decreased capacity to enhance second-phase insulin release (Fig. 5, bottom panel), despite a comparable or slightly lower  $EA_{50}$  value (measure of drug sensitivity).

## Discussion

GLP-1 receptor agonists represent a new class of insulinotropic agents that are being evaluated for their potential as therapeutic agents for the treatment of type 2 diabetes (Doyle and Egan, 2001; Doyle and Egan, 2003). To our knowledge, this study is the first instance of applying a mechanistic model to quantify the dynamics of exendin-4 under hyperglycemic clamp conditions. The likely mechanism of action of exendin-4 was integrated into an existing model of the glucose-insulin system, thereby facilitating the simultaneous analysis of glucose and insulin responses to hyperglycemia and drug effects. Drug concentrations could not be obtained in the previous study as the available Amylin radioimmunoassay at that time lacked the necessary sensitivity for the administered dosing regimen. In the absence of measured drug concentrations, an inverse PD modeling approach was utilized, with PK driving functions that are approximated during the process of modeling response profiles (Gabrielsson et al., 2000).

In general, the final estimated parameters controlling glucose and insulin regulation (Table 1) are in accordance with most literature values (Bergman et al., 1979; Weber et al., 1989; Neatpisarnvanit and Boston, 2002; Pillonetto et al., 2002; Agerso and Vicini, 2003; Osterberg et al., 2003), as would be expected from the MAP Bayesian estimation approach used herein. However, some discrepancies were evident. For example, the linear first-order uptake of glucose ( $p_1$ ) and insulin ( $n$ ) are typically greater in nondiabetic subjects as compared to diabetic subjects (approximately 2-fold). In addition, the threshold glucose concentrations ( $h$ ) should be in close proximity to baseline measures ( $G_0$ ), whereas the estimated value in healthy subjects appears underestimated. Sources of these inconsistencies are varied and can range from significant inter-subject variability (among individuals within the same group) to model complexity and/or misspecification. With regards to the latter, systematic appraisals of the minimal model have

## JPET #69765

yielded cautionary statements involving model assumptions, especially when applications are intended to provide clinical assessments of patient conditions (Avogaro et al., 1996; Mari, 1997; Cobelli et al., 1998). For our purposes, however, the model structure provided a suitable framework in which to incorporate exendin-4 PK/PD properties, and the final model is proposed for such analyses. Furthermore, the use of Bayesian estimation has been shown to effectively deal with the modeling difficulties associated with the structure of the minimal model (Pillonetto et al., 2003).

In a previous study, the single point plasma concentrations of NN2211 (a different GLP-1 analogue) at the time of administering an IV glucose tolerance test were found to correlate with several of the model parameters ( $T_{sec}$ ,  $I_{rel}$ , and  $\gamma$ ) (Agerso and Vicini, 2003). Effects on  $T_{sec}$  and  $I_{rel}$  would not be observed in this study as the first-phase insulin release was completed long before exendin-4 was administered (simulations of Eq. 4 show that  $IR_1$  is essentially zero by the time the exendin-4 infusion is started; data not shown). The final model of NN2211 included a linear relationship between the single point drug concentrations and the effective  $\gamma$  parameter ( $\gamma_{eff}$ ). Our final model is thus a logical extension based on these observations and the mechanisms of action of GLP-1 agonists (Doyle and Egan, 2001). Although  $E_{max}$  values were approximately 2-fold lower in diabetic volunteers, relative stimulatory capacity ( $E_{max}/\gamma$ ) or apparent efficacy is similar ( $6.75 \pm 1.89$  vs.  $6.31 \pm 0.76$  for healthy and type 2 diabetic subjects; mean  $\pm$  s.e.). Effective PK functions were approximated using a simple one-compartment model with linear first-order elimination (Eq. 6). Interestingly, a previous PK study of exendin-4 reported that drug concentrations following an IV infusion (0.028 pmol/kg/min) decreased with first-order kinetics and a half-life of  $26 \pm 3$  minutes (Edwards et al., 2001). That would correspond with a mean terminal elimination rate of  $0.0267 \text{ min}^{-1}$  ( $\ln(2)/T_{1/2}$ ) and is similar to our

## JPET #69765

estimates of  $k_e$  (Table 1). Finally, the overall impact of the estimated pharmacological properties of exendin-4 was ascertained via PK/PD simulations (Fig. 5), revealing a reduced capacity for diabetic subjects to release insulin in response to exendin-4, despite comparable PK ( $k_e$ ), sensitivity ( $EA_{50}$ ), and efficacy ( $E_{\max}/\gamma$ ) parameters, which makes sense intuitively.

In conclusion, a mechanistic PD model was developed that provides quantitative insights into the in vivo PK/PD properties of exendin-4 in nondiabetic and type 2 diabetic subjects, extracted from glucose and insulin response profiles. Although future studies that include measured drug concentrations are needed, this inverse PD modeling study suggests that exendin-4 exposure and efficacy may be similar between these populations; however, diabetic subjects still exhibit a decreased capacity for insulin release as a function of their baseline system parameters. This model may prove useful in future clinical studies of other GLP-1 derivatives that employ the hyperglycemic clamp technique.

JPET #69765

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JPET #69765

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JPET #69765

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JPET #69765

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JPET #69765

### **Footnotes**

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JPET #69765

## Figure legends

- Fig. 1.** Physiological pharmacodynamic model of exendin-4 effects on glucose and insulin regulation during controlled hyperglycemia. Symbols are defined under PD Model in Methods.
- Fig. 2.** Mean plasma glucose (top panels) and insulin (bottom panels) concentrations following a hyperglycemic clamp (0-300 min) and an exendin-4 infusion (solid bars; 60-120 min) in nondiabetic (**A**) and type 2 diabetic (**B**) subjects. The insets in the bottom panels show first-phase insulin release on an adjusted scale. Error bars represent standard error (n=7). Adapted from Egan et al. (2002).
- Fig. 3.** Representative plots of model (Fig. 1) fitted profiles (solid lines) in one nondiabetic (**A**) and one type 2 diabetic (**B**) subject. Solid bars show the duration of the exendin-4 infusions.
- Fig. 4.** Model predicted and observed glucose (top panels) and insulin (bottom panels) concentrations in the nondiabetic (**A**) and diabetic (**B**) groups. Each panel contains lines of unity (dashed) and linear regression (solid).
- Fig. 5.** Simulated effective exendin-4 exposure (top panel) and  $\gamma_{eff}$  values (bottom panel) as a function of time in nondiabetic (solid lines) and diabetic (dashed lines) populations using mean parameter estimates (Table 1). Solid bars show the duration of the exendin-4 infusions. Equations are defined in text.

**Table 1.** Mean estimated model parameters following a 60-minute intravenous infusion of exendin-4 in healthy and type 2 diabetic subjects under hyperglycemic clamp conditions

Parameter (units)	Healthy Subjects			Type 2 Diabetic Subjects		
	Prior	Mean	CV%	Prior	Mean	CV%
$T_{dur}$ (min)	1.9	1.71	7.1	1.9	1.76	7.3
$T_{sec}$ (min)	3.5	3.41	8.6	3.5	3.54	20
$I_{rel}$ ( $\mu\text{U}/\text{mL}$ )	NI	49.9	85	NI	13.0 <sup>a</sup>	199
$V$ (dL/kg)	1.3	1.39	20	1.3	1.42	14
$p_1$ ( $\text{min}^{-1}$ )	$3.0 \times 10^{-2}$	$2.28 \times 10^{-2}$	20	$1.3 \times 10^{-2}$	$2.09 \times 10^{-2}$	35
$p_2$ ( $\text{min}^{-1}$ )	$3.0 \times 10^{-2}$	$3.39 \times 10^{-2}$	57	$1.3 \times 10^{-2}$	$1.61 \times 10^{-2a}$	47
$p_3$ ( $\text{min}^{-2}/\mu\text{U}/\text{mL}$ )	$7.50 \times 10^{-6}$	$5.60 \times 10^{-6}$	52	$3.0 \times 10^{-6}$	$1.49 \times 10^{-6}\dagger$	72
$\gamma$ ( $\mu\text{UmL}^{-1}\text{min}^{-2}/\text{mgdL}^{-1}$ )	$2.8 \times 10^{-4}$	$5.52 \times 10^{-4}$	43	$3.0 \times 10^{-4}$	$2.43 \times 10^{-4*}$	76
$h$ (mg/dL)	98	63.2	28	137	144 $\dagger$	40
$n$ ( $\text{min}^{-1}$ )	0.21	0.165	41	0.13	0.204	26
$E_{max}$ ( $\mu\text{UmL}^{-1}\text{min}^{-2}/\text{mgdL}^{-1}$ )	NI	$2.81 \times 10^{-3}$	55	NI	$1.54 \times 10^{-3a}$	86
$EA_{50}$ (pmol/kg)	NI	0.268	64	NI	0.111 <sup>a</sup>	69
$k_e$ ( $\text{min}^{-1}$ )	NI	$3.86 \times 10^{-2}$	50	NI	$4.60 \times 10^{-2}$	32
$\sigma_G$	N/A	0.137	37	N/A	$5.02 \times 10^{-2}\dagger$	62
$\sigma_I$	N/A	0.433	55	N/A	0.146 <sup>*</sup>	26

N/A, not applicable; NI, non-informative

<sup>a</sup> Trend for lower values than for healthy subjects ( $p = 0.081, 0.057, 0.12,$  and  $0.058$  for  $I_{rel}, p_2, E_{max},$  and  $EA_{50}$  respectively).

\* Significantly different from healthy subjects ( $p < 0.05$ ).

† Significantly different from healthy subjects ( $p \leq 0.01$ ).

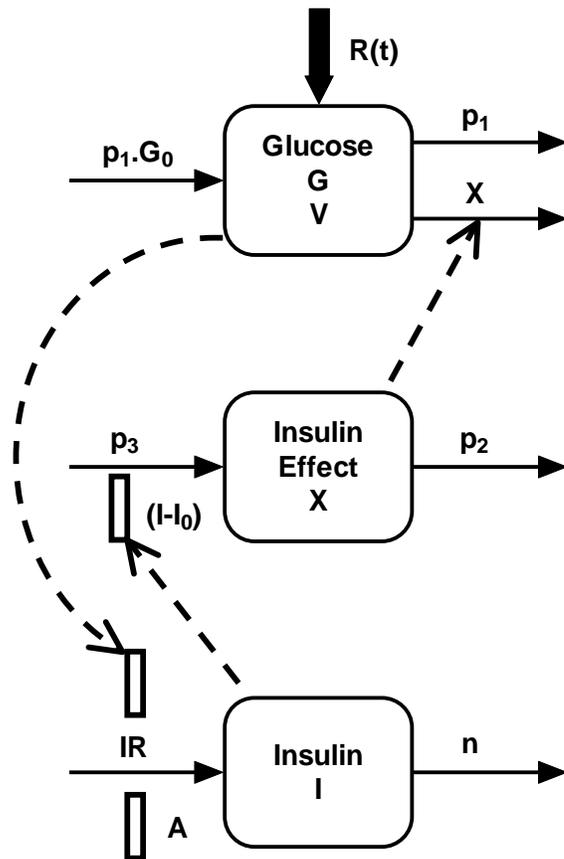


Fig. 1

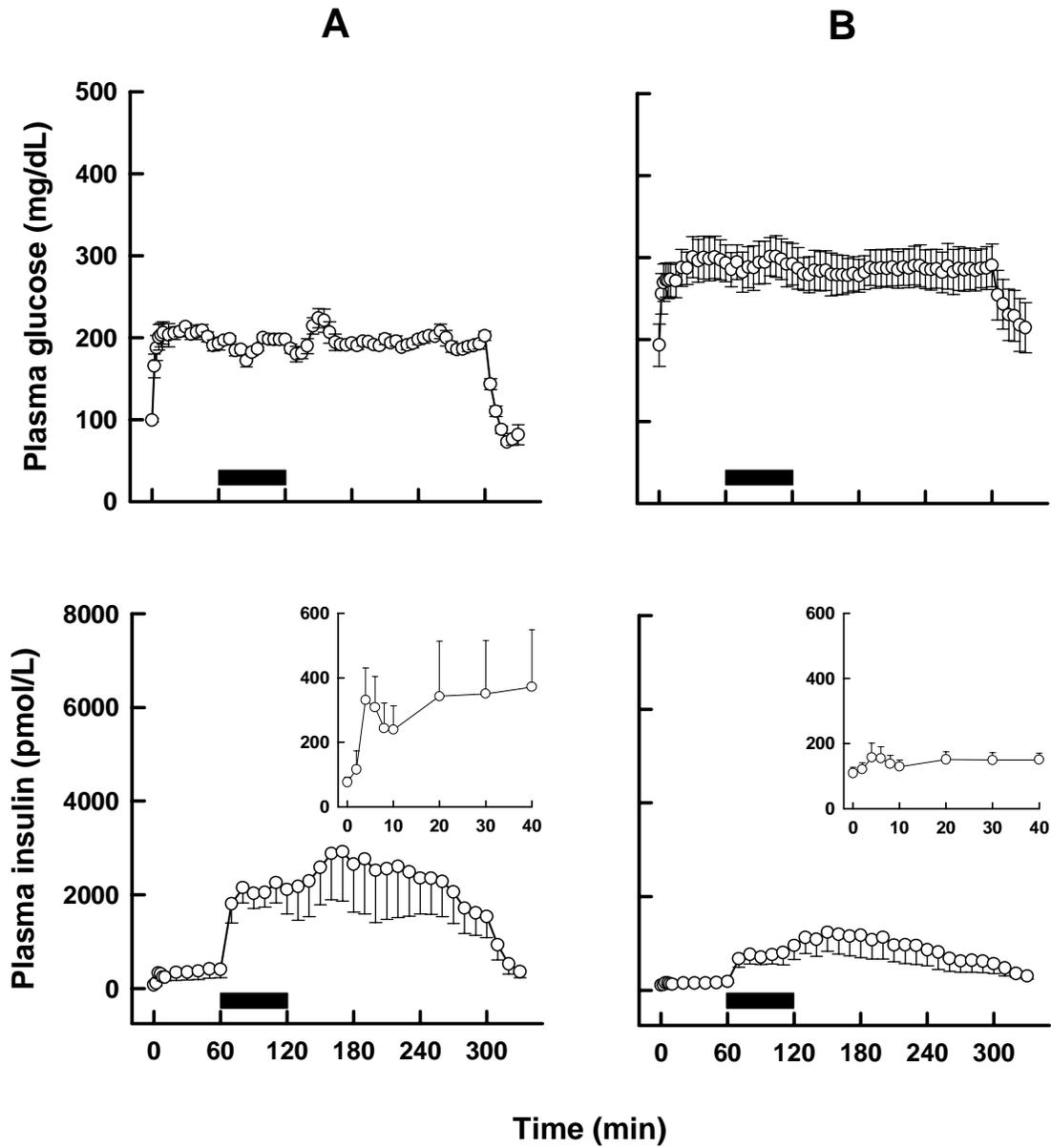


Fig. 2

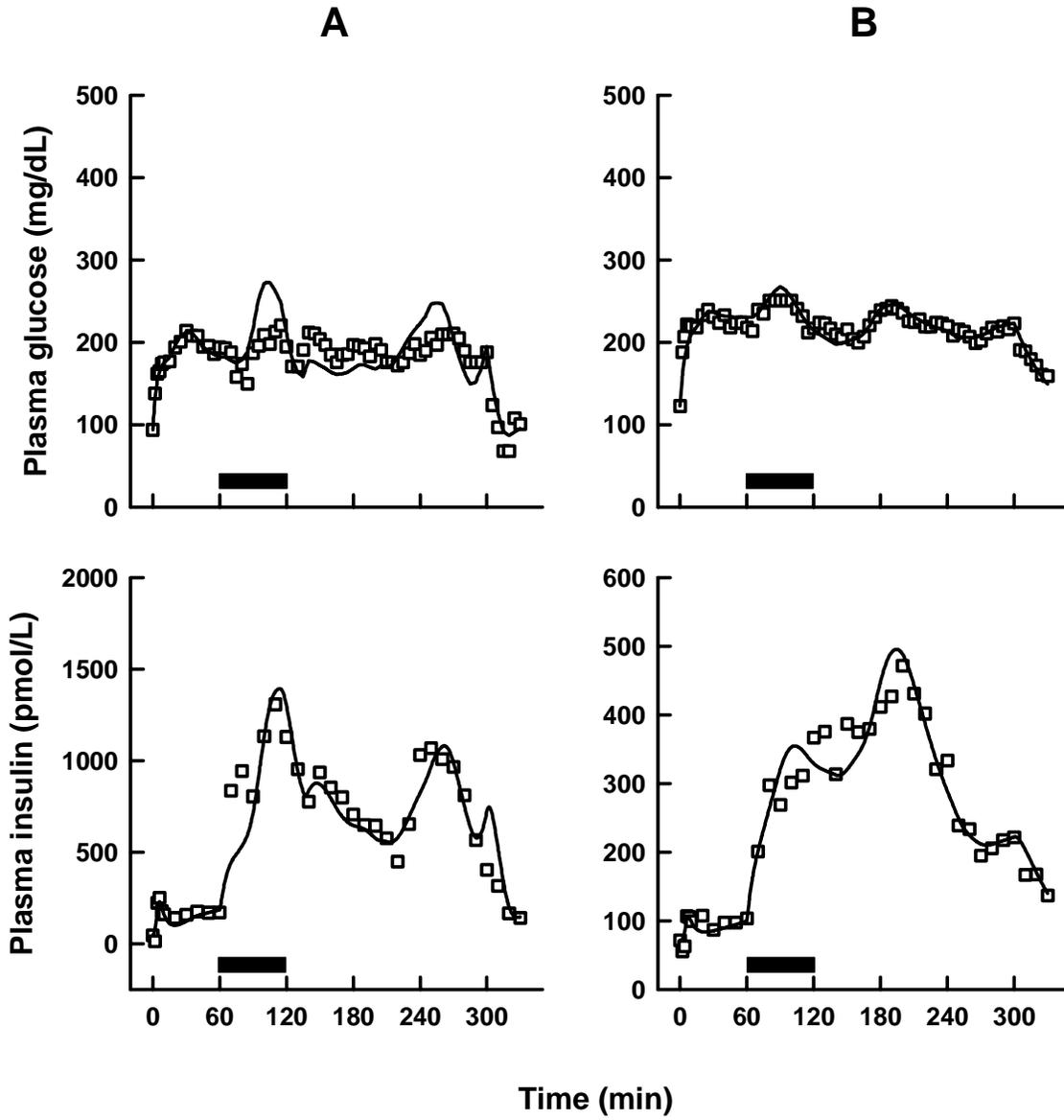


Fig. 3

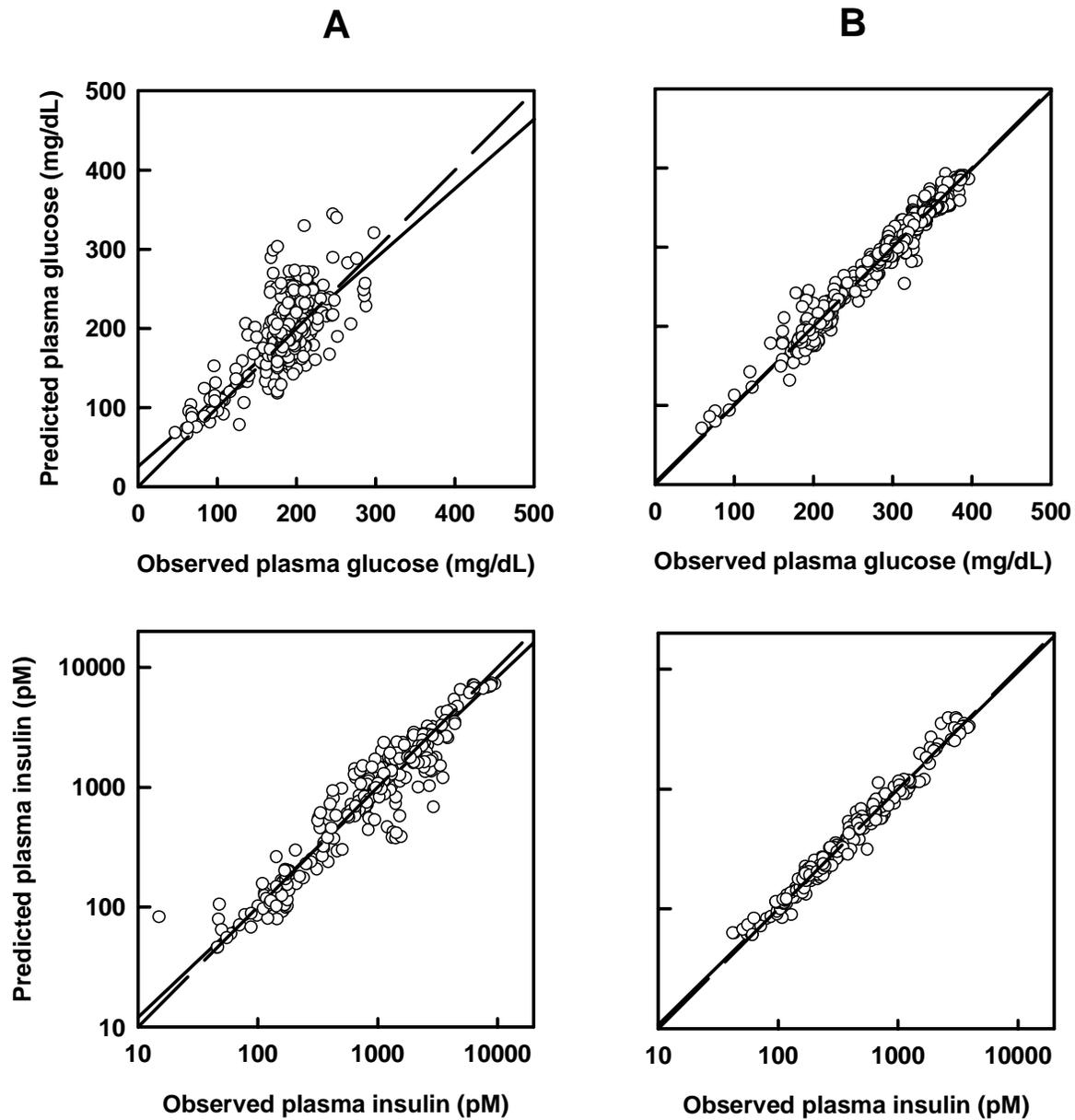


Fig. 4

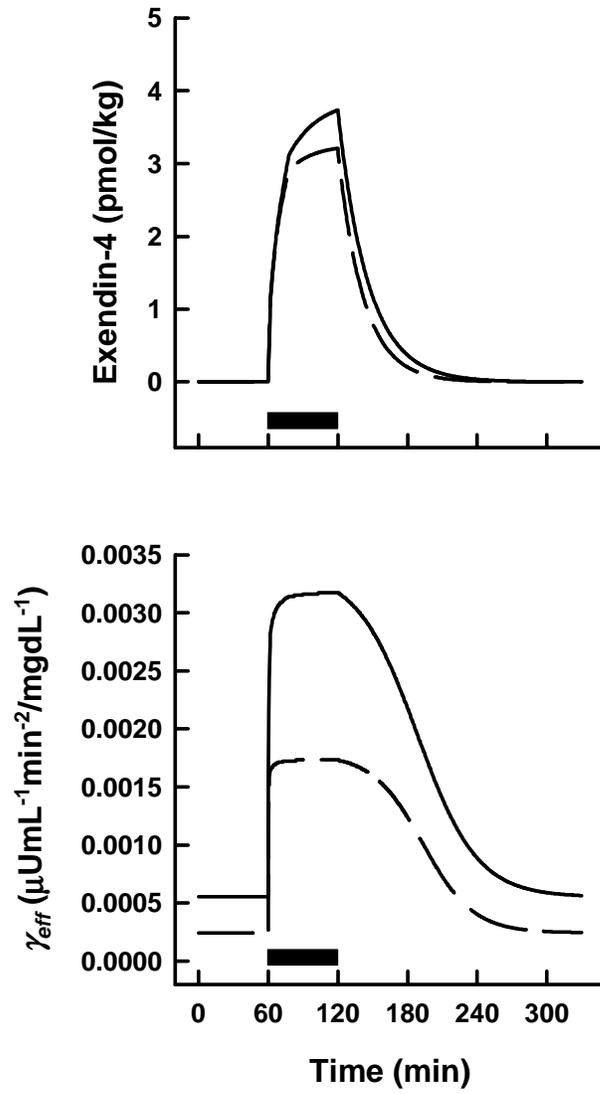


Fig. 5