

Target-mediated drug disposition modeling of liraglutide in rats: Implications of target engagement for pharmacodynamic predictions

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ABSTRACT

Target-mediated drug disposition (TMDD) is a pharmacokinetic phenomenon in which high-affinity binding to specific receptors leads to nonlinear pharmacokinetics. This study evaluated the pharmacokinetics and pharmacodynamics of the GLP-1 receptor agonist liraglutide in normal and diabetic rats, and developed a TMDD model to mechanistically link receptor dynamics to pharmacological outcomes. Liraglutide was administered intravenously across a wide dose range (0.005–0.8 mg/kg in normal rats; 0.005–0.2 mg/kg in diabetic rats). Plasma concentrations of liraglutide were determined using validated LC–MS/MS methods. Noncompartmental analysis revealed dose-dependent increases in clearance and volume of distribution at lower doses, consistent with TMDD behavior. A TMDD model adequately captured the observed concentration–time profiles across all dose levels. In diabetic rats, the pharmacodynamic response, measured as blood glucose reduction, exhibited a saturating relationship with both dose and plasma exposure (AUC). In contrast, the individually predicted AUC of the drug–receptor complex ($AUC_{D-R \text{ complex}}$) showed a linear correlation with the area under the effect curve (AUEC; $\beta = 0.93$, 95% CI 0.64–1.21), suggesting that receptor occupancy may serve as a more relevant determinant of pharmacodynamic response than plasma drug concentration. Simulations further revealed dose-dependent receptor depletion and saturable drug–receptor complex formation, providing mechanistic explanations for the prolonged pharmacological effects and nonlinear exposure–response relationship of GLP-1 receptor agonists. These findings support the utility of TMDD modeling for linking receptor dynamics to pharmacodynamic outcomes and provide a translational framework for rational dose optimization of peptide therapeutics.

1. Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists are therapeutic agents that mimic the action of endogenous GLP-1, an incretin hormone that plays a critical role in glucose homeostasis [1,2]. GLP-1 is secreted from intestinal L cells in response to nutrient intake, where it slows gastric emptying, stimulates glucose-dependent insulin release, and suppresses glucagon secretion [3]. However, endogenous GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), resulting in a plasma half-life of only approximately 2 min [4]. To overcome this limitation, synthetic GLP-1 receptor (GLP-1R) agonists have been developed with structural modifications conferring enhanced stability and extended duration of action [5]. Currently approved GLP-1R agonists include exenatide, liraglutide, and semaglutide, as well as GLP-1R/GIPR dual agonists such as tirzepatide.

Liraglutide is a GLP-1 analog with 97% sequence homology to native

GLP-1. It contains a C₁₆ fatty acid side chain that enables albumin binding, reduces renal clearance, and provides resistance to DPP-4 degradation. These modifications result in a half-life of approximately 13 h, allowing once-daily subcutaneous dosing [6–8]. Liraglutide paved the way to more patient-friendly management of type 2 diabetes mellitus (T2DM) and obesity with a synthetic GLP-1R agonist. Beyond glucose control, large clinical outcome trials have also demonstrated cardiovascular and renal benefits [9,10].

In drug development, PK characterization provides the basis for dose optimization, efficacy prediction, and safety evaluation. While many drugs exhibit linear PK, nonlinear PK is frequently observed, in which systemic exposure is not directly proportional to dose [11,12]. Such nonlinear behavior may result from saturable absorption, protein binding, metabolism, or diverse elimination pathways. Among these mechanisms, target-mediated drug disposition (TMDD) is particularly relevant to therapeutic peptides and biologics [13]. TMDD occurs when

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drugs bind with high affinity to a limited-capacity pharmacological target, leading to dose-dependent clearance that is most pronounced at low concentrations [14–16].

Nonlinear PK behavior, including TMDD, complicates the determination of appropriate dose regimens and hinders accurate prediction of drug elimination at low concentrations [17]. Moreover, dose or exposure alone often fails to adequately explain pharmacodynamic (PD) responses. For drugs that follow TMDD, target-binding events underlie the molecular processes governing PD outcomes. Mechanism-based mathematical modeling can therefore provide quantitative insight into such target dynamics.

Previous studies have reported nonlinear PK for early GLP-1 analogs such as exendin-4 [18] and exenatide [19], suggesting possible involvement of TMDD for other GLP-1 analogs. The pharmacokinetic behavior of liraglutide in humans has been well characterized following subcutaneous administration at the approved therapeutic dose range, demonstrating dose-proportional exposure [20–23]. However, systematic characterization of dose-dependent nonlinear disposition across a wide dose range, and the extent to which TMDD contributes, has not been reported [24]. Moreover, while TMDD modeling has been extensively applied to monoclonal antibodies, its application to therapeutic peptides such as GLP-1 receptor agonists remains limited [17,25].

Therefore, the present study aimed to investigate the dose-dependent PK of liraglutide in rats and to mechanistically characterize its nonlinear disposition using a TMDD model. Pharmacodynamic (PD) responses were further evaluated in diabetic rats to explore their relationship with PK. Incorporating receptor dynamics and drug-receptor complex kinetics revealed an otherwise inaccessible relationship between exposure and effect. Ultimately, the developed TMDD model offers a preclinical framework that may inform rational dose selection of GLP-1R agonists by linking receptor occupancy to pharmacodynamic response.

2. Materials and methods

2.1. Materials

Liraglutide was obtained from Chengdu Shengnuo Biopharm Co., Ltd (Chengdu, China). The internal standard (IS) semaglutide was obtained from Zhejiang Peptides Biotech Co., Ltd. (Hangzhou, China). Formic acid was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and water were obtained from J.T. Baker Co. (Philipsburg, NJ, USA).

2.2. In vivo studies

2.2.1. Animals

Male Sprague-Dawley (SD) rats were purchased from Samtako Bio Korea (Osan, Korea). Rats in the normal group were 8 weeks of age at the time of the study (body weight: 0.261 ± 0.030 kg). Rats designated for the diabetic group were obtained at 6 weeks of age to allow for the diabetes induction period. All rats were housed individually under controlled environmental conditions (temperature: 23 ± 3 °C; humidity: $55 \pm 10\%$; 12 h light/dark cycle) with free access to water and either a standard diet (Youngbio, Seongnam, Korea) for the normal group or a high-fat diet (Research Diets, Inc., New Brunswick, NJ, USA) for the diabetic group. The animal studies were approved by the Institutional Animal Care and Use Committee (SKKUIACUC2025–06–29–1) and conducted in accordance with the standard operating procedures.

2.2.2. Induction of type-2 diabetes

To prepare model animals with T2DM (the diabetic group), a combination of a high-fat diet and streptozotocin (STZ) injections was used. Following a two-week period of a high-fat diet (45 kcal% fat), the animals received two injections of STZ administered three days apart. Freshly prepared 20 mg/mL STZ in 0.1 M sodium citrate buffer (pH =

4.5) was administered intraperitoneally at a dose of 35 mg/kg. Body weight and blood glucose levels were monitored during the T2DM induction period to monitor animal welfare and ensure survival. The blood glucose levels were measured from tail vein blood using a portable glucometer (GC Medical Science Corp., Yongin, Korea). Rats with fasting blood glucose levels exceeding 350 mg/dL were considered diabetic. T2DM-induced rats weighed 0.281 ± 0.013 kg at the time of drug administration.

2.2.3. Pharmacokinetic and pharmacodynamic studies

Rats were fasted for at least 16 h prior to drug administration. For the pharmacokinetic studies of liraglutide in normal rats, rats were randomly assigned to eight groups and received intravenous (IV) injections at doses of 0.8, 0.4, 0.2, 0.1, 0.05, 0.02 ($n = 4$ per group), 0.01, or 0.005 mg/kg ($n = 3$ per group). Each dosing solution was prepared by dissolving liraglutide in normal saline at a concentration corresponding to the intended dose (0.8–0.005 mg/mL). Intravenous injection was selected to explicitly characterize the disposition of liraglutide, avoiding the confounding variability introduced by absorption after subcutaneous administration.

For diabetic rats, rats were randomly assigned to four groups and received IV injections of liraglutide at doses of 0.2, 0.05, 0.01 ($n = 6$ per group), or 0.005 mg/kg ($n = 8$ per group). The dose range was selected to capture the TMDD-relevant concentration range identified in normal rats, allowing examination of whether a similar nonlinear disposition occurs in the diabetic state and how receptor occupancy relates to the glucose-lowering effect. The larger group sizes in diabetic rats were employed to account for greater expected variability and concurrent pharmacodynamic measurements. Blood samples (0.3 mL) were collected from the jugular vein at predefined time points post-dose. Plasma was separated by centrifugation at $3220 \times g$ for 10 min at 4 °C and stored at -20 °C until analysis.

Pharmacodynamic assessment was conducted only in diabetic rats, as liraglutide exerts glucose-dependent effects with minimal impact on normoglycemic animals [26]. Blood glucose levels in diabetic rats were measured at 30, 15, and 0 min pre-dose, and at each PK sampling time point, using a portable glucometer (GC Medical Science Corp.). The blood glucose level at each time point was determined as the average value of triplicate measurements. The baseline glucose level of each animal was defined as the average of its three pre-dose measurements. The coefficient of variation (CV) of the baseline glucose level was calculated as the standard deviation divided by the mean of the pooled pre-dose measurements across all diabetic rats [27].

2.3. LC–MS/MS

Plasma concentrations of liraglutide were analyzed using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods [28–30]. Liraglutide was quantified using either an Agilent 6490 triple-quadrupole mass spectrometer coupled with an Agilent 1260 HPLC (Agilent Technologies, Santa Clara, CA, USA), or a SCIEX QTrap 6500+ triple-quadrupole mass spectrometer (SCIEX, Framingham, MA, USA) coupled to an Agilent 1290 HPLC system. The Agilent 6490 system was used for normal group samples, while the SCIEX QTrap 6500+ system was used for diabetic group samples. Chromatographic separations were performed on an Acquity™ Premier Peptide CSH C18 column (150×2.1 mm, i.d., $1.7 \mu\text{m}$, Waters, Milford, MA, USA), using gradient elution with mobile phases consisting of formic acid in distilled water (A) and formic acid in acetonitrile (B). Formic acid content was 0.3% for the Agilent 6490 system and 0.1% for the SCIEX QTrap 6500+ system. The electrospray ionization (ESI) source was operated in positive ion mode. The selected m/z transitions for multiple reaction monitoring (MRM) analysis were $938.5 \rightarrow 1064$ for liraglutide, and $1029.4 \rightarrow 1302.9$ for semaglutide (internal standard).

The assay was validated in accordance with FDA guidelines for bioanalytical method validation [31]. The lower limit of quantification

(LLOQ) was 2 ng/mL (0.53 nM) with the Agilent 6490 system and 0.5 ng/mL (0.13 nM) with the SCIEX QTrap 6500+ system.

2.4. Pharmacokinetic and pharmacodynamic analyses

2.4.1. Noncompartmental pharmacokinetic analysis

Noncompartmental analysis (NCA) was used to evaluate the pharmacokinetic parameters of liraglutide. Key parameters included terminal half-life ($t_{1/2}$), initial concentration (C_0), area under the plasma concentration–time curve from time zero to the last observation time point (AUC_{all}) and infinity (AUC_{inf}), clearance (CL), the volume of distribution at steady state (V_{ss}), and the mean residence time (MRT).

2.4.2. Pharmacodynamic analysis

The ‘effect’ was defined as the relative decrease (%) in blood glucose level from the individual baseline:

$$\text{Effect (\%)} = \frac{\text{Baseline} - \text{Observed}}{\text{Baseline}} \times 100 \quad (1)$$

The baseline was defined as the average of each animal's pre-dose measurements. To determine whether a measured change was physiologically meaningful, the reference change value (RCV) was calculated as [32]:

$$\text{RCV} = \sqrt{2} \times Z \times \text{CV} \quad (2)$$

where Z is the Z-score (1.96 for 95% significance). Based on the observed CV of baseline glucose measurements, the RCV was 9.84%, and a 10% cutoff was adopted to minimize noise from baseline fluctuations when calculating AUEC. The area under the effect curve (AUEC) was calculated using the trapezoidal rule with data up to 48 h to ensure adequate data density for reliable estimation. The onset time, defined as the time at which the effect first reached 10%, was estimated by linear interpolation. The same approach was used to estimate the duration of effect above 20% (D_{20}). Among these PD outcomes, AUEC was used as the primary metric for PK–PD correlation, as it integrates both the magnitude and duration of the pharmacodynamic response.

2.4.3. Model development

A population pharmacokinetic model incorporating target-mediated drug disposition (TMDD) was developed to characterize the pharmacokinetics of liraglutide. A one-compartment model was used to describe the disposition of liraglutide, consistent with previous findings [21] and the monoexponential decline observed in the present study. The overall pharmacokinetic model structure is illustrated in Fig. 1. The model consists of three components, i.e., drug, receptor, and drug–receptor complex. Drug elimination follows a first-order process characterized by the elimination rate constant, k_{el} . Receptor dynamics are described by a zero-order production rate constant, k_{in} , and a first-order degradation rate constant, k_{out} . At steady state, the baseline receptor concentration R_{base} is determined by the ratio k_{in}/k_{out} . Drug–receptor binding is governed by the association rate constant (k_{on}) and

dissociation rate constant (k_{off}), with the dissociation constant, K_d , being k_{off}/k_{on} . The model estimated k_{el} , R_{base} , k_{out} , K_d , and k_{off} directly, while k_{in} and k_{on} were calculated from the above relationships.

The differential equations for the drug amount (X_{drug}) in the central compartment and the free receptor concentration ($C_{receptor}$) are as follows:

$$\frac{dX_{drug}}{dt} = -k_{el} \cdot X_{drug} - k_{on} \cdot X_{drug} \cdot C_{receptor} + k_{off} \cdot C_{D-R \text{ complex}} \cdot V \quad (3)$$

$$\frac{dC_{receptor}}{dt} = k_{in} - k_{out} \cdot C_{receptor} - k_{on} \cdot \left(\frac{X_{drug}}{V}\right) \cdot C_{receptor} + k_{off} \cdot C_{D-R \text{ complex}} \quad (4)$$

$$k_{in} = R_{base} \cdot k_{out} \quad (5)$$

The drug–receptor complex ($C_{D-R \text{ complex}}$) is eliminated via internalization or degradation, described by the first-order rate constant k_m . The differential equation for the complex concentration is given as follows:

$$\frac{dC_{D-R \text{ complex}}}{dt} = k_{on} \cdot \left(\frac{X_{drug}}{V}\right) \cdot C_{receptor} - k_{off} \cdot C_{D-R \text{ complex}} - k_m \cdot C_{D-R \text{ complex}} \quad (6)$$

$$k_{on} = k_{off} / K_D \quad (7)$$

Parameter sets for the normal and diabetic groups were separately estimated using S-ADAPT® software. Between-subject variability (BSV) was accounted for using an exponential error model. Model performance was assessed by comparing observed concentrations with both individual- and population-predicted values (goodness-of-fit), providing insight into model fitness and potential prediction bias.

To evaluate the predictive performance of the developed pharmacokinetic model, a thousand Monte Carlo simulations were conducted using Berkeley Madonna software for each dose level. Visual predictive check (VPC) plots were generated to compare the observed plasma concentration vs. time profiles with the corresponding 5th–95th percentile prediction intervals. In addition, the average values of AUC_{inf} , AUC_{inf}/dose , CL, and V_{ss} of the simulations were compared with the observed values, and prediction errors were evaluated as follows:

$$\text{PE (\%)} = \frac{|\text{Predicted} - \text{Observed}|}{\text{Observed}} \times 100 \quad (8)$$

Finally, the developed pharmacokinetic model was employed to simulate the temporal profiles of free receptor and drug–receptor complex concentrations as well as the area under the complex concentration–time curve ($AUC_{D-R \text{ complex}}$). These simulations were conducted using Berkeley Madonna software across the dosing range used in the *in vivo* studies to examine how receptor dynamics and complex formation varied with dose.

2.5. Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD) unless otherwise indicated. Pharmacokinetic parameters obtained by non-compartmental analysis were compared between the normal and diabetic groups at each corresponding dose level using Student's *t*-test. Statistical significance was set at $p < 0.05$.

A power model was applied to quantitatively assess linearity between two parameters. The relationship between the log-transformed values of each parameter was evaluated by linear regression:

$$\ln X = \ln \alpha + \beta \cdot Y \quad (9)$$

Linear correlation was concluded when the 95% confidence interval (CI) of the estimated slope (β) included the value 1. A β greater than 1 indicates a more-than-proportional increase, while a β less than 1 indicates a less-than-proportional increase.

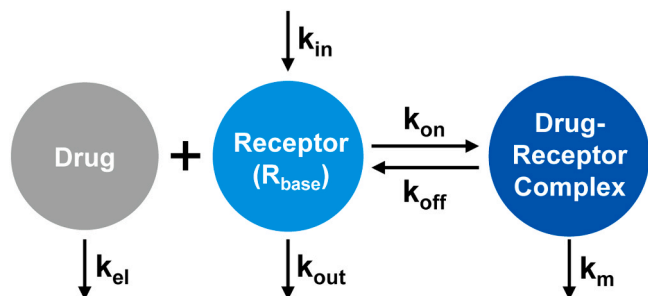


Fig. 1. Schematic representation of the pharmacokinetic model with target-mediated drug disposition (TMDD) for liraglutide.

3. Results

3.1. Pharmacokinetics of liraglutide in rats

3.1.1. Pharmacokinetics in normal rats

Figs. 2A and 2B show the average plasma concentration vs. time profiles of liraglutide following intravenous injection at eight dose levels (0.005–0.8 mg/kg) in normal rats. The corresponding non-compartmental pharmacokinetic parameters of liraglutide are summarized in Table 1.

The elimination half-life ($t_{1/2}$) ranged from 3.16 to 5.26 h across doses. The average clearance (CL) was highest at the lowest dose ($23.07 \text{ mL}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ at 0.005 mg/kg) and decreased with increasing dose, reaching a plateau at doses over 0.2 mg/kg. Similarly, the steady-state volume of distribution (V_{ss}) also exhibited a dose-dependent decrease, from $103.47 \text{ mL}\cdot\text{kg}^{-1}$ at 0.005 mg/kg to approximately $45 \text{ mL}\cdot\text{kg}^{-1}$ at doses over 0.2 mg/kg. The dose-dependent changes in CL and V_{ss} at lower doses are indicative of TMDD-driven nonlinear pharmacokinetics.

3.1.2. Pharmacokinetics in diabetic rats

Figs. 2C and 2D present the plasma concentration–time profiles of liraglutide at four dose levels (0.005–0.2 mg/kg) in diabetic rats. Non-compartmental pharmacokinetic parameters are listed in Table 1. As observed in normal rats, both CL and V_{ss} decreased with increasing dose, from $22.92 \text{ mL}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ and $108.21 \text{ mL}\cdot\text{kg}^{-1}$ at 0.005 mg/kg to $9.14 \text{ mL}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ and $54.81 \text{ mL}\cdot\text{kg}^{-1}$ at 0.2 mg/kg, respectively, indicating that TMDD behavior is preserved in diabetic rats. When NCA parameters were compared at each corresponding dose level, no statistically significant differences were detected between the normal and diabetic groups ($p > 0.05$ for all comparisons).

3.1.3. Assessment of dose proportionality

Both normal and diabetic rats exhibited dose-dependent decreases in dose-normalized AUC_{inf} ($\text{AUC}_{\text{inf}}/\text{Dose}$) and increases in CL and V_{ss} at lower doses, consistent with nonlinear pharmacokinetics (Fig. 3). At higher doses, these parameters approached constant values, indicating linear pharmacokinetic behavior.

To quantitatively assess dose proportionality, a power model was applied to the relationship between AUC_{inf} and dose. When all dose levels were included, β was significantly greater than 1 ($\beta = 1.25$, 95% CI: 1.22–1.28), indicating deviation from dose proportionality (Fig. 4A). The lowest doses were then sequentially excluded, and β remained above 1 until the analysis was restricted to doses $\geq 0.2 \text{ mg/kg}$, where dose proportionality was established ($\beta = 1.08$, 95% CI: 0.97–1.19) (Fig. 4B). The complete results of the sequential analysis are provided in Supplementary Table S1.

3.2. Pharmacodynamics of liraglutide in diabetic rats

Diabetic rats, with a mean baseline blood glucose level of 500 mg/dL (CV: 3.55%), exhibited significant glucose reduction at all dose levels (Fig. 5A). Blood glucose levels declined rapidly after dosing, reached a nadir between 2 and 6 h, and gradually returned toward baseline within 48 h. The magnitude and duration of glucose reduction were dose-dependent, although the incremental response diminished at higher doses, suggesting a saturable pharmacodynamic relationship. The pharmacodynamic outcomes at each dose are summarized in Table 2. The maximum effect (E_{max}), the time of maximum effect ($T_{\text{max},E}$), the duration above 20% effect (D_{20}), and AUEC all increased with dose, but in a less than dose-proportional manner.

Fig. 5B illustrates the contrasting behavior of PK and PD with respect to dose. AUEC increased less than proportionally with dose and saturated at higher doses, whereas AUC_{inf} increased more than proportionally across the same dose range. This divergence suggests that plasma

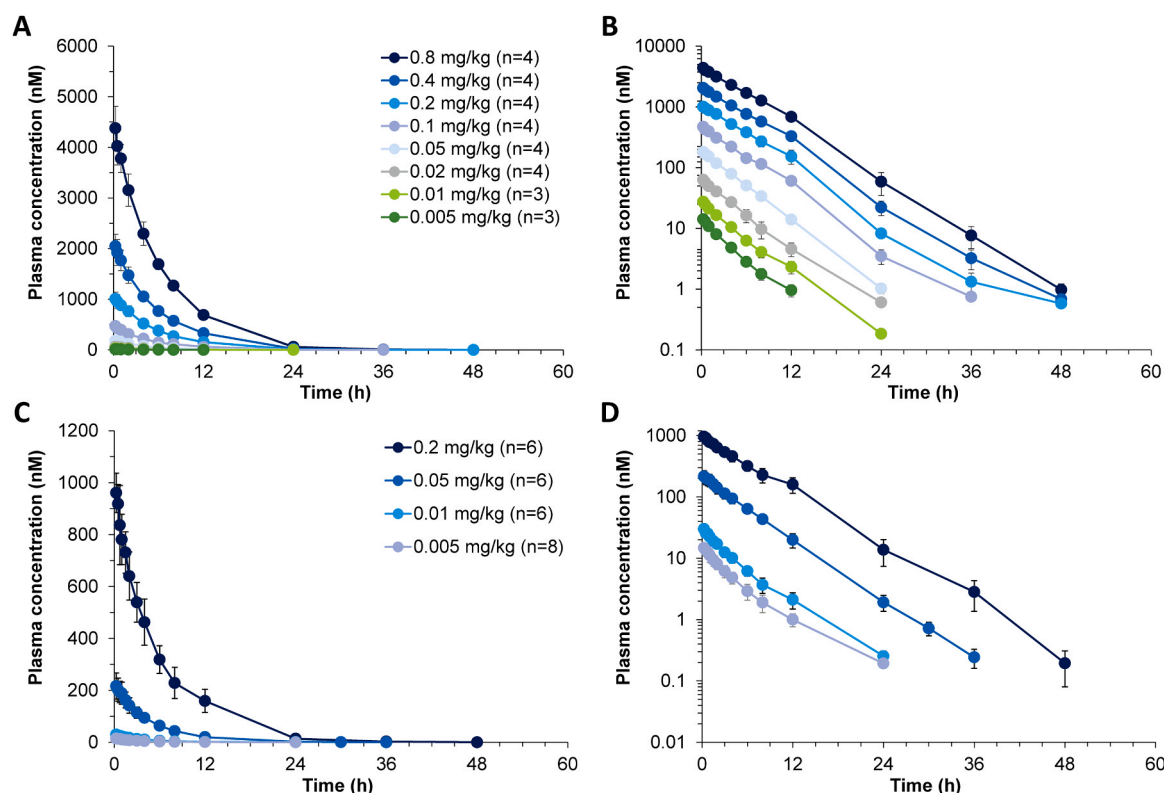


Fig. 2. Average plasma concentration vs. time profiles of liraglutide following intravenous injection in normal rats at doses 0.8, 0.4, 0.2, 0.1, 0.05, 0.02 ($n = 4$), 0.01, and 0.005 ($n = 3$) mg/kg presented on linear (A) and semi-logarithmic (B) scales, and in diabetic rats at doses 0.2, 0.05, 0.01 ($n = 6$), and 0.005 ($n = 8$) mg/kg presented on linear (C) and semi-logarithmic (D) scales.

Table 1
Noncompartmental pharmacokinetic parameters of liraglutide following intravenous injection at various doses in normal (n = 3–4) and diabetic (n = 6–8) rats (mean ± SD).

Group	Dose (mg/kg)	$t_{1/2}$ (h)	C_0 (ng/mL)	AUC_{all} (ng·h/mL)	AUC_{inf} (ng·h/mL)	CL ($mL·h^{-1}·kg^{-1}$)	V_{ss} ($mL·kg^{-1}$)	MRT (h)
Normal rats	0.005	3.77 ± 0.43	57.93 ± 0.69	199.42 ± 21.84	218.98 ± 26.39	23.07 ± 2.97	103.47 ± 6.39	4.48 ± 0.38
	0.01	3.87 ± 0.46	113.73 ± 20.20	440.66 ± 33.20	471.81 ± 23.03	21.23 ± 1.05	103.79 ± 8.74	4.89 ± 0.43
	0.02	3.42 ± 0.41	258.24 ± 14.90	1075.94 ± 198.05	1115.36 ± 177.27	18.31 ± 3.16	81.20 ± 4.10	4.50 ± 0.51
	0.05	3.16 ± 0.11	739.96 ± 86.02	3399.17 ± 323.03	3416.77 ± 325.82	14.74 ± 1.43	67.86 ± 6.21	4.61 ± 0.11
	0.1	4.43 ± 0.55	1942.71 ± 160.98	10,148.90 ± 634.51	10,187.20 ± 629.35	9.84 ± 0.58	53.41 ± 3.94	5.43 ± 0.20
	0.2	5.26 ± 1.81	3989.92 ± 523.73	24,393.22 ± 3466.69	24,413.65 ± 3470.72	8.32 ± 1.25	45.94 ± 4.63	5.55 ± 0.40
	0.4	4.51 ± 0.98	8184.95 ± 841.87	50,064.58 ± 4415.78	50,086.13 ± 4417.25	8.03 ± 0.73	46.54 ± 4.38	5.79 ± 0.22
	0.8	4.15 ± 0.37	17,889.40 ± 955.85	108,272.20 ± 4962.70	108,294.16 ± 4966.69	7.40 ± 0.33	43.53 ± 4.45	5.88 ± 0.44
Diabetic rats	0.005	4.04 ± 0.56	60.38 ± 9.26	215.87 ± 60.67	228.47 ± 53.98	22.92 ± 5.08	108.21 ± 24.53	4.74 ± 0.41
	0.01	3.63 ± 0.61	134.51 ± 49.82	464.54 ± 81.62	471.39 ± 80.42	21.72 ± 3.61	92.81 ± 28.00	4.62 ± 0.68
	0.05	3.70 ± 0.35	897.83 ± 238.35	4222.49 ± 676.40	4236.53 ± 671.70	12.07 ± 2.02	60.87 ± 11.20	5.04 ± 0.43
	0.2	3.64 ± 0.42	3777.78 ± 336.42	22,415.89 ± 4108.76	22,422.62 ± 4107.58	9.14 ± 1.45	54.81 ± 6.86	6.04 ± 0.47

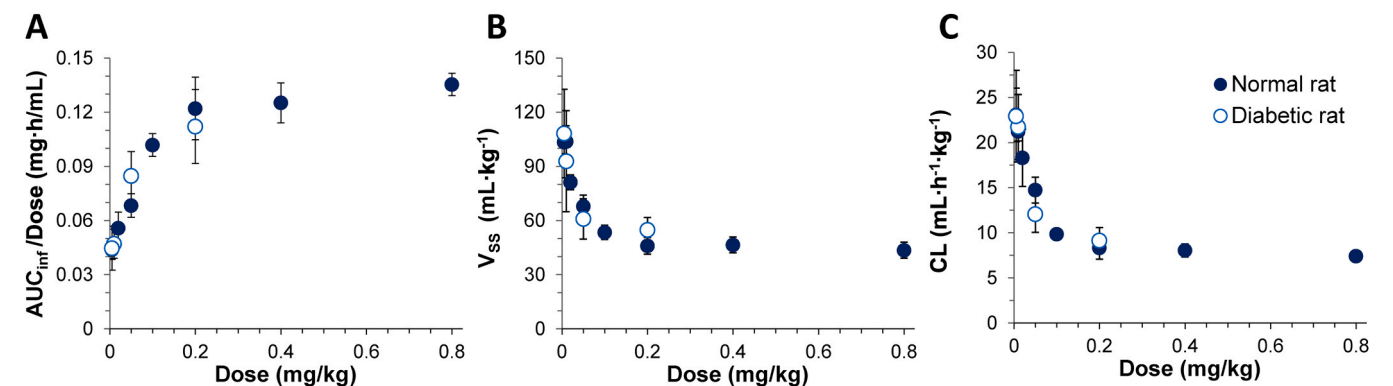


Fig. 3. Dose-dependent changes in pharmacokinetic parameters of liraglutide in normal and diabetic rats: dose-normalized AUC_{inf} ($AUC_{inf}/Dose$) (A), steady-state volume of distribution (V_{ss}) (B), and clearance (CL) (C).

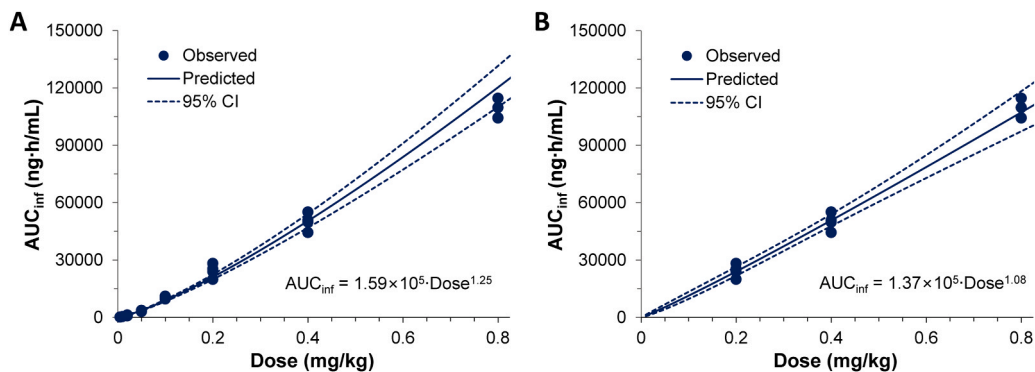


Fig. 4. Assessment of dose proportionality of AUC_{inf} for liraglutide in normal rats using a power model. (A) All dose levels (0.005–0.8 mg/kg): AUC_{inf} increased more than proportionally with dose ($\beta = 1.25$). (B) Doses ≥ 0.2 mg/kg: AUC_{inf} was proportional to dose ($\beta = 1.08$).

drug exposure alone does not fully account for liraglutide's pharmacodynamic response.

3.3. Development of the TMDD model

A population pharmacokinetic model incorporating TMDD was developed to characterize the nonlinear pharmacokinetics of liraglutide (Fig. 1). Plasma concentration–time data across all dose levels were simultaneously fitted for each group. The population PK parameter estimates for both the normal and diabetic rats are summarized in Table 3. In both groups, the estimated V_d and k_{el} values were comparable to the NCA-derived V_{ss} and CL/V_{ss} at the highest dose, where linear pharmacokinetics predominated (Table 1). This agreement supports the

appropriateness of the one-compartment model with TMDD to describe the pharmacokinetic profiles of liraglutide.

Model validation was performed by 1000 Monte Carlo simulations for each dose level. The VPC plots showed that most observed concentrations fell within the 5th–95th percentile prediction intervals, confirming that the TMDD model adequately captured the observed pharmacokinetic profiles of liraglutide across all dose levels in both groups (Fig. 6). Goodness-of-fit plots also showed data points evenly distributed around the line of identity for both individual and population predictions (Fig. 7). To further assess predictive performance, PK parameters were derived from the simulated concentration–time profiles using NCA and compared with those obtained from the observed data. Prediction errors were below 30% for all parameters and dose

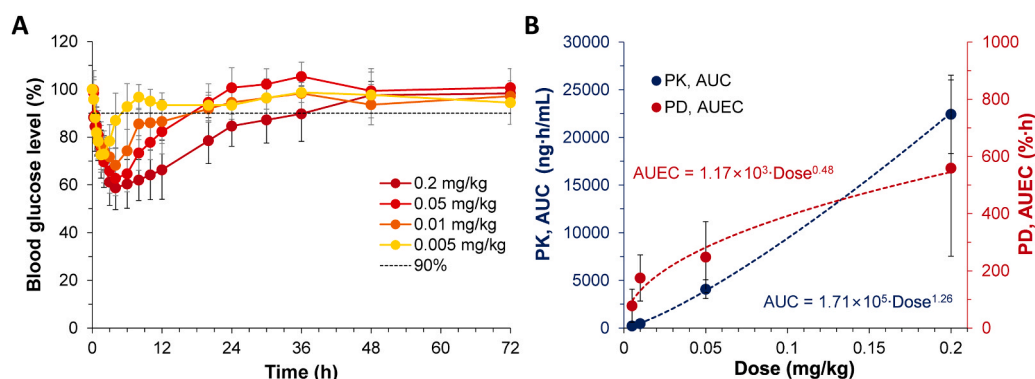


Fig. 5. Pharmacodynamics of liraglutide in diabetic rats. (A) Time course of blood glucose levels (% of baseline) at each dose level. (B) Dose-dependent changes in AUC_{inf} and AUEC. Data are presented as mean $\pm$ SD ($n = 6-8$).

Table 2

Pharmacodynamic outcomes of liraglutide in diabetic rats (mean $\pm$ SD, $n = 6-8$).

Dose (mg/kg)	E_{max} (%)	$T_{max,E}$ (h)	D_{20} (h)	AUEC (%·h)
0.2	42.88 ± 8.40	6.00 ± 3.35	20.94 ± 10.29	559.37 ± 307.98
0.05	39.60 ± 6.87	4.33 ± 1.37	9.82 $\pm$ 4.31	248.16 ± 124.08
0.01	32.00 ± 6.90	4.00 ± 1.10	7.72 $\pm$ 4.98	175.15 $\pm$ 80.51
0.005	28.89 ± 6.82	1.94 ± 0.50	2.71 $\pm$ 1.82	77.99 $\pm$ 58.06

levels (Table 4), confirming that the model accurately reproduced the observed PK characteristics across the entire dose range. The largest PE (V_{ss} at 0.005 mg/kg in normal rats, 55%) occurred at the lowest dose, where TMDD-driven nonlinearity is most pronounced, and does not indicate systematic model misspecification. Overall, these results confirm adequate model performance with no apparent systematic bias.

Consistent with the NCA results, the model-estimated disposition parameters (V_d and k_{el}) were comparable between normal and diabetic rats. The model further indicated differences in receptor-related parameters that were not apparent from NCA alone. R_{base} in diabetic rats (12.7 nM) was approximately half that of normal rats (26.2 nM), and K_d was higher in the diabetic group (4.73 vs. 3.45 nM), suggesting potential alteration of receptor dynamics under diabetic conditions.

Table 3

Population pharmacokinetic parameter estimates of liraglutide in normal and diabetic rats.

Parameter	Symbol	Unit	Population mean (BSV%)			
			Normal	SE%	Diabetic	SE%
Volume of distribution	V_d	$\text{mL} \cdot \text{kg}^{-1}$	55.4 (12%)	2.37% (29.1%)	62.5 (14%)	2.99% (29.8%)
Elimination rate constant of drug	k_{el}	h^{-1}	0.159 (7%)	2.18% (60.2%)	0.166 (12%)	6.71% (97.1%)
Baseline concentration of receptor	R_{base}	nM	26.2 (16%)	6.70% (57.8%)	12.7 (23%)	8.12% (66.2%)
Production rate constant of receptor	k_{in}	nM	3.50 (-) ^a	-	2.65 (-) ^a	-
Degradation rate constant of receptor	k_{out}	h^{-1}	0.133 (49%)	14.2% (41.9%)	0.209 (48%)	27.0% (136%)
Dissociation constant	K_d	nM	3.45 (5%)	10.9% (155%)	4.73 (17%)	22.1% (99.8%)
Association rate constant	k_{on}	$\text{nM}^{-1} \cdot \text{h}^{-1}$	0.041 (-) ^b	-	0.068 (-) ^b	-
Dissociation rate constant	k_{off}	h^{-1}	0.143 (2%)	2.22% (193%)	0.323 (3%)	21.0% (154%)
Elimination rate constant of drug-receptor complex	k_{in}	h^{-1}	0.088 (12%)	7.69% (187%)	0.122 (20%)	23.6% (64.5%)

^a, k_{in} was calculated from the model-estimated population mean values as $k_{in} = k_{out} \times R_{base}$

^b, k_{on} was calculated from the model-estimated population mean values as $k_{on} = k_{off} / K_d$

3.4. Prediction of receptor occupancy and pharmacodynamic response

Using the validated TMDD model, temporal profiles of free receptor and drug-receptor complex were simulated across the dose range (Fig. 8). Free receptor availability showed dose-dependent depletion (Fig. 8A). At low doses (0.005–0.1 mg/kg), receptors were only partially occupied and recovered relatively rapidly. At higher doses (0.2–0.8 mg/kg), the receptor pool was nearly fully depleted and remained suppressed for a prolonged period. Drug-receptor complex concentrations increased with dose, although the incremental gain diminished at higher doses (Fig. 8B), and the corresponding $AUC_{D-R \text{ complex}}$ showed a less-than-proportional increase with dose (Fig. 8C).

To identify the determinant of pharmacodynamic response, individual AUEC values were plotted against dose, AUC_{inf} , and individually predicted $AUC_{D-R \text{ complex}}$ (Fig. 9). AUEC exhibited saturating, nonlinear relationships with both dose ($\beta = 0.51$, 95% CI: 0.34–0.67) and AUC_{inf} ($\beta = 0.39$, 95% CI: 0.26–0.53). In contrast, AUEC and $AUC_{D-R \text{ complex}}$ demonstrated a near-linear correlation ($\beta = 0.93$, 95% CI: 0.64–1.21), suggesting that receptor occupancy may more closely reflect pharmacodynamic response than plasma concentration.

4. Discussion

The present study characterized the nonlinear pharmacokinetics of liraglutide using a TMDD model and explored the link between receptor dynamics and pharmacodynamic response. Liraglutide exhibited TMDD-driven nonlinear pharmacokinetics, which was systematically

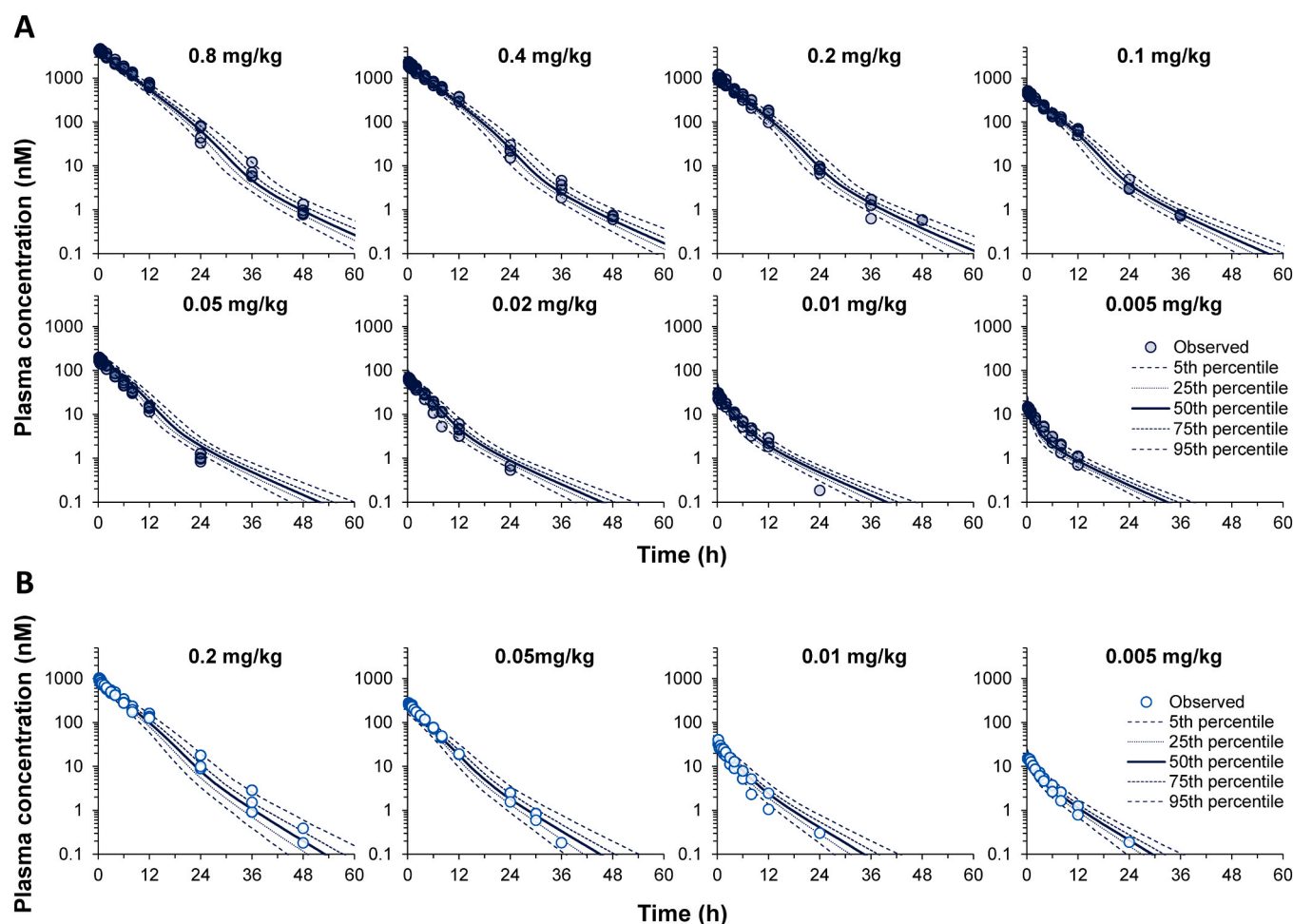


Fig. 6. Visual predictive check plots for the plasma concentration–time profiles at 0.005–0.8 mg/kg in normal rats (A) and 0.005–0.2 mg/kg in diabetic rats (B). Lines represent the 5th to 95th percentile prediction intervals from 1000 Monte Carlo simulations, and circles represent observed data.

characterized across a wide dose range. Beyond capturing these non-linearities, the TMDD model indicated that the drug–receptor complex may more closely reflect the glucose-lowering effect (Fig. 9). This demonstrates that TMDD modeling not only explains pharmacokinetics but also provides a mechanistic link to pharmacodynamic outcomes.

The pharmacokinetic parameters of liraglutide obtained in this study are consistent with the previous reports following IV injection in rats [28]. In those studies, however, only a single dose (0.05 mg/kg) was evaluated, and nonlinear pharmacokinetics could not be characterized. The present study examined a wide dose range (0.005–0.8 mg/kg), enabling clear identification of dose-dependent changes in CL and V_{ss} . Power model analysis confirmed that dose proportionality was achieved only at doses ≥ 0.2 mg/kg, below which receptor-mediated clearance becomes a significant elimination pathway. Nonlinear pharmacokinetics driven by TMDD have been previously reported for other GLP-1 analogs such as exendin-4 and exenatide [18,19]. The present study provides systematic evidence that liraglutide follows the same disposition mechanism.

The estimated TMDD parameters provide further mechanistic insight. R_{base} was 26.2 nM in normal rats, which is about five times greater than the values reported for exendin-4 or exenatide (both ~ 5.2 nM) [18,19]. The discrepancy decreased when comparing the total amount ($R_{base} \times V_d$) rather than the concentration (1.45 nmol/kg versus 0.6 and 0.73 nmol/kg for exendin-4 and exenatide in normal rats, respectively). The estimated dissociation constant ($K_d = 3.45$ nM) was close to the apparent *in vitro* GLP-1R binding IC_{50} value in the presence of 2% HSA (4.78 nM) [33]. Since liraglutide extensively binds to

albumin, both values represent apparent affinities that account for protein binding, supporting the physiological plausibility of the model estimate [34]. Although the internalization rate of the GLP-1R itself is very fast ($t_{1/2} = 2\text{--}3$ min, corresponding to $k_m = 14\text{--}21$ h^{−1}) [35], the model estimated a much smaller k_m value. This is expected, as the model estimate represents macroscopic target-mediated clearance rather than cellular-level receptor internalization.

Although there was no significant difference in NCA parameters between normal and diabetic rats, TMDD modeling indicated potential differences in receptor-related parameters between the two groups. The estimated R_{base} in diabetic rats (12.7 nM) was approximately half that in normal rats (26.2 nM), which is consistent with reduced GLP-1R expression reported under hyperglycemic conditions [36,37]. The diabetic group also exhibited a higher K_d (4.73 vs. 3.45 nM), suggesting reduced binding affinity, and a higher k_m (0.122 vs. 0.088 h^{−1}), consistent with faster internalization. Under diabetic pathophysiological conditions, GLP-1R dynamics can shift towards rapid internalization driven by phosphorylation-dependent mechanisms [36,38]. Such post-translational modifications may also affect receptor–ligand interactions, consistent with the higher K_d observed in the diabetic group. These findings illustrate the potential of TMDD modeling to identify disease-related changes in receptor dynamics that are not apparent from conventional analyses.

The simulation of receptor dynamics provided mechanistic insights that are not accessible from plasma concentration–time data alone. The dose-dependent depletion of free receptors and the saturable formation of drug–receptor complex explain the saturation of pharmacodynamic

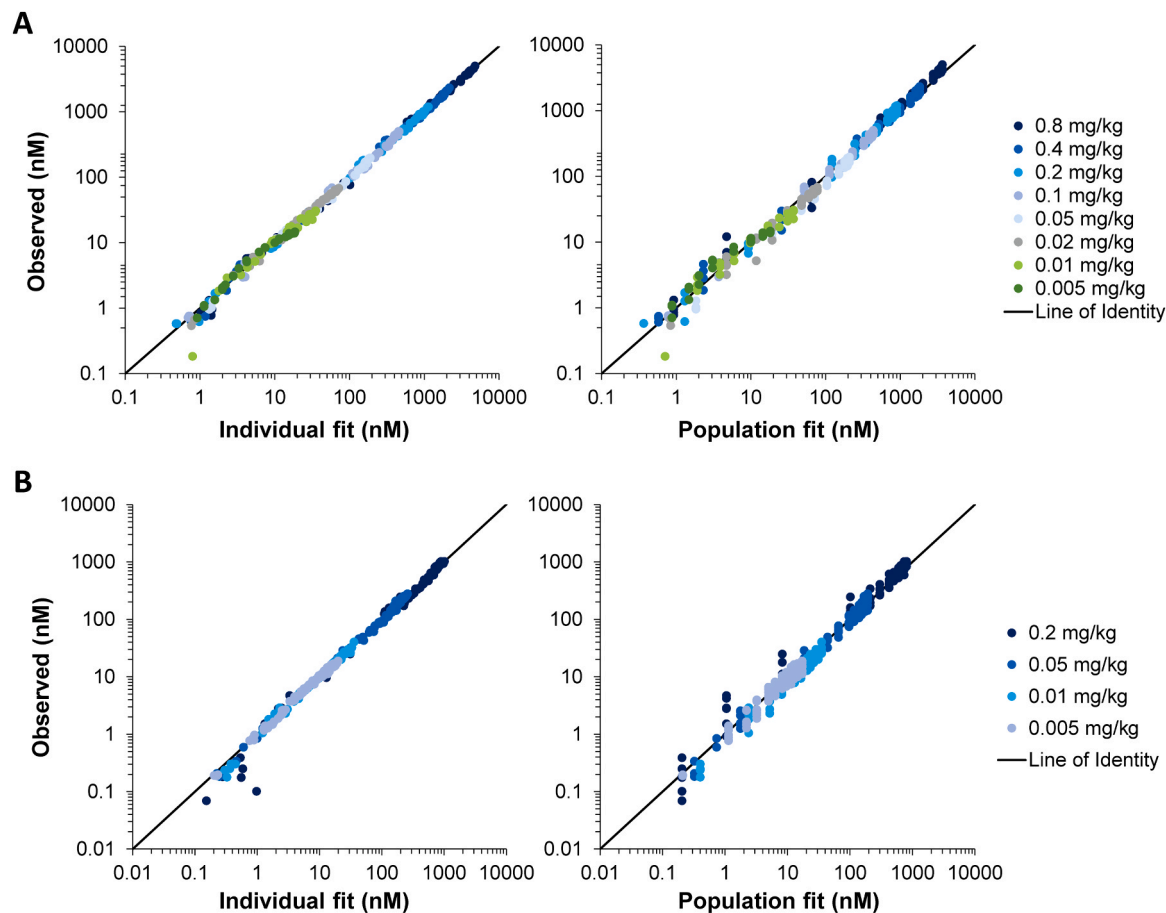


Fig. 7. Goodness-of-fit plots for the population pharmacokinetic model in normal rats (A) and diabetic rats (B). Observed versus individual-predicted (left) and population-predicted (right) concentrations are shown. The line represents the line of identity.

Table 4
Comparison of the observed and model-predicted pharmacokinetic parameters of liraglutide in normal and diabetic rats.

Group	Dose (mg/kg)	AUC _{inf} (ng·h/mL)			V _{ss} (mL·kg ⁻¹)			CL (mL·h ⁻¹ ·kg ⁻¹)		
		Observed	Predicted	PE (%)	Observed	Predicted	PE (%)	Observed	Predicted	PE (%)
Normal rats	0.8	108,294.16 ± 4966.69	88,656.76	18.13	43.53 ± 4.45	54.31	24.77	7.40 ± 0.33	9.20	24.29
	0.4	50,086.13 ± 4417.25	42,819.62	14.51	46.54 ± 4.38	54.71	17.55	8.03 ± 0.73	9.52	18.58
	0.2	24,413.65 ± 3470.72	20,607.18	15.59	45.94 ± 4.63	54.78	19.23	8.32 ± 1.25	9.92	19.21
	0.1	10,187.20 ± 629.35	9684.15	4.94	53.41 ± 3.94	56.04	4.93	9.84 ± 0.58	10.58	7.52
	0.05	3416.77 ± 325.82	4245.25	24.25	67.86 ± 6.21	62.01	8.62	14.74 ± 1.43	12.13	17.68
	0.02	1115.36 ± 177.27	1315.39	17.93	81.20 ± 4.10	82.72	1.87	18.31 ± 3.16	15.87	13.32
	0.01	471.81 ± 23.03	495.01	4.92	103.79 ± 8.74	119.08	14.73	21.23 ± 1.05	21.12	0.52
	0.005	218.98 ± 26.39	199.01	9.12	103.47 ± 6.39	160.25	54.88	23.07 ± 2.97	26.19	13.51
Diabetic rats	0.2	22,422.62 ± 4107.58	17,929.05	20.04	54.81 ± 6.86	62.00	13.12	9.14 ± 1.45	11.53	26.19
	0.05	4236.53 ± 671.7	3991.16	5.79	60.87 ± 11.2	65.99	8.41	12.07 ± 2.02	13.02	7.94
	0.01	471.39 ± 80.42	580.64	23.18	92.81 ± 28	94.00	1.28	21.72 ± 3.61	18.23	16.06
	0.005	228.47 ± 53.98	248.72	8.86	108.21 ± 24.53	115.11	6.38	22.92 ± 5.08	21.21	7.48

response at higher doses: once receptor binding sites are saturated, additional drug is eliminated through non-specific pathways without contributing to receptor engagement. AUC_{D-R complex} and AUEC demonstrated a near-linear correlation, in contrast to the saturating relationships observed with dose or AUC_{inf}. AUC_{D-R complex} can be interpreted as the cumulative signal generated by agonist binding to GLP-1R over time. All downstream processes, including glucose-dependent insulin secretion, glucagon suppression, and subsequent counter-regulatory responses, are initiated by this receptor engagement. The overall glucose-lowering effect, estimated as AUEC, is therefore expected to be proportional to AUC_{D-R complex} rather than to plasma drug concentration. This finding supports the notion that receptor occupancy

rather than systemic exposure may be a more relevant determinant of the pharmacodynamic response of GLP-1R agonists. Although receptor occupancy was not directly determined in this study, in vitro binding characteristics of liraglutide at the GLP-1R are well established, and direct in vivo quantification requires specialized experimental approaches that were beyond the scope of the present study. The TMDD modeling framework provides a practical alternative, and the close agreement between observed pharmacokinetic data and model predictions supports the reliability of the simulated receptor dynamics. Model-based estimation of receptor occupancy is particularly valuable when direct measurement is not feasible, as is often the case for membrane-bound receptors such as GLP-1R.

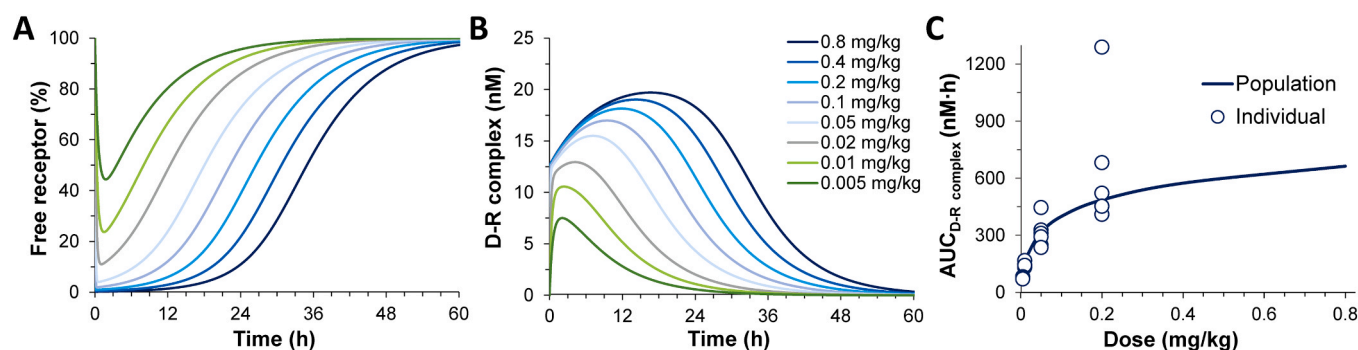


Fig. 8. Prediction of free receptor occupancy and drug–receptor complex formation. Fraction of free receptor (A) and drug–receptor complex concentration (B) were simulated across the dose range of 0.005–0.8 mg/kg using population mean parameter estimates. (C) Population mean $AUC_{D-R \text{ complex}}$ at each dose compared with individually predicted values.

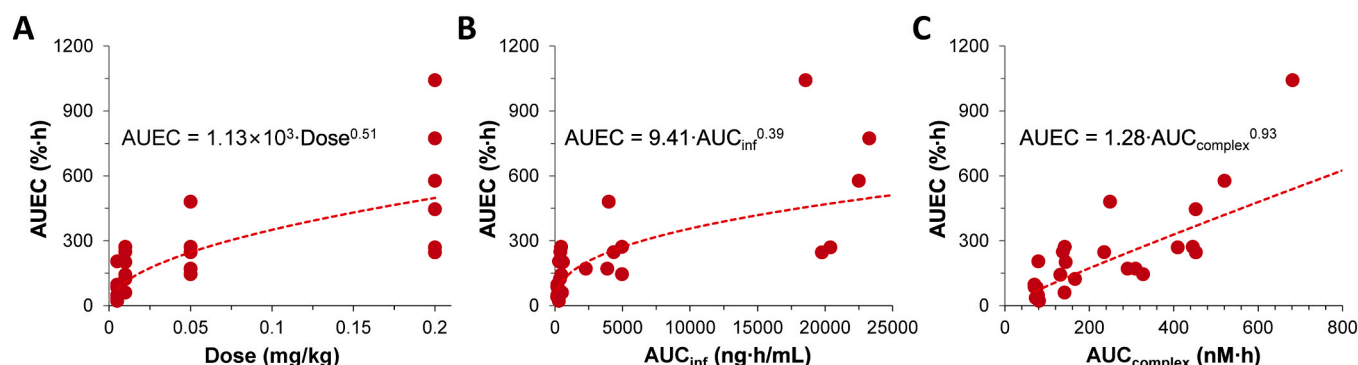


Fig. 9. Correlation of AUEC with (A) dose, (B) AUC_{inf} , and (C) individually predicted $AUC_{D-R \text{ complex}}$ in diabetic rats. Dashed lines represent power model regression fits. β and 95% CI are indicated for each correlation.

The apparent dose-proportionality observed in human studies [20–23] likely reflects receptor binding site saturation at established therapeutic concentrations, rendering the nonlinear component imperceptible at the systemic level. Species differences in receptor density, binding characteristics, and albumin binding may further contribute, and direct extrapolation between species remains challenging [39]. However, the therapeutic indications for GLP-1 receptor agonists continue to expand, and novel analogs with altered receptor binding characteristics and new formulations are actively under development. In these contexts, the mechanistic characterization of TMDD behavior across a wide concentration range becomes particularly important.

Nonlinear pharmacokinetics complicates dose selection and exposure prediction, particularly at low concentrations where receptor-mediated clearance becomes a dominant elimination pathway. In patient populations where receptor availability or binding characteristics may differ, plasma concentration-based monitoring alone may therefore be insufficient to predict pharmacodynamic outcomes. In clinical settings, receptor occupancy modeling has guided dose selection for monoclonal antibodies, and a similar approach may be applicable to GLP-1R agonists. The modeling approach described here may therefore contribute to more informed dose optimization strategies. Furthermore, as TMDD behavior has been reported for other GLP-1 receptor agonists, the modeling approach described here may serve as a translational framework applicable to the broader development of this therapeutic class. Further studies incorporating subcutaneous administration and additional pharmacodynamic endpoints would strengthen the translational value of this framework.

5. Conclusions

In conclusion, this study demonstrated that liraglutide exhibits

TMDD-driven nonlinear pharmacokinetics in rats, which was successfully characterized by a population TMDD model across a wide dose range. The model further indicated that the drug–receptor complex may serve as a closer surrogate for pharmacodynamic response compared with plasma drug concentration. These findings support the utility of TMDD modeling as a framework for linking receptor dynamics to therapeutic outcomes of GLP-1R agonists.

CRedit authorship contribution statement

Sungmin Song: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Joonhee Kim:** Investigation, Formal analysis. **Siyeon Kim:** Investigation. **Beom Soo Shin:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Soyoung Shin:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Soyoung Shin reports financial support was provided by Ministry of Trade, Industry & Resources, Republic of Korea. Beom Soo Shin reports financial support was provided by National Research Foundation of Korea. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biopha.2026.119345](https://doi.org/10.1016/j.biopha.2026.119345).

Data availability

All data generated or analyzed during this study are included in this published article and the supplementary files.

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