The Effects of a GLP-1 Analog on Glucose Homeostasis in Type 2 Diabetes Mellitus Quantified by an Integrated Glucose Insulin Model

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In recent years, several glucagon-like peptide-1 (GLP-1)-based therapies for the treatment of type 2 diabetes mellitus (T2DM) have been developed. The aim of this work was to extend the semimechanistic integrated glucose-insulin model to include the effects of a GLP-1 analog on glucose homeostasis in T2DM patients. Data from two trials comparing the effect of steady-state liraglutide vs. placebo on the responses of postprandial glucose and insulin in T2DM patients were used for model development. The effect of liraglutide was incorporated in the model by including a stimulatory effect on insulin secretion. Furthermore, for one of the trials an inhibitory effect on glucose absorption was included to account for a delay in gastric emptying. As other GLP-1 receptor agonists have similar modes of action, it is believed that the model can also be used to describe the effect of other receptor agonists on glucose homeostasis.

RESULTS

A total of 29 T2DM patients, from two trials (referred to as trial 1 and trial 2 throughout the article), were included in the pharmacokinetic-pharmacodynamic (PK-PD) analysis and detailed descriptions of the two trials are available in Flint et al.14 and Hermansen et al.15 PK measurements of liraglutide were available from both studies, with sparse sampling in trial 1 (4 samples per subject at each dose level) and frequent sampling in trial 2 (12 samples per subject at the highest dose level). The PD measurements, i.e., plasma glucose and serum insulin, measuring glucose homeostasis effects, as well as paracetamol, measuring gastric emptying effects, were sampled for 5 hours in trial 1 and 8 hours in trial 2 after MTTs in the presence of placebo or study drug. Treatment duration was 3 weeks in both trials. The liraglutide/placebo dose was escalated weekly in 0.6 mg increments from 0.6 mg until a daily dose of 1.8 mg was reached. In trial 1 MTTs were performed at each dose level, whereas in trial 2 MTTs were only performed at the highest dose level. In both trials standardized meals were served. In trial 2, a high-fat meal with a caloric density of 4 MJ was served, and in trial 1 the caloric density of the meal was 2 MJ.

The incretin hormone glucagon-like peptide-1 (GLP-1), which is secreted from the small intestine following food intake, is a potent glucose-lowering agent. The glucoregulatory effects of GLP-1 are facilitated through enhanced glucose-stimulated insulin secretion, slowed gastric emptying, glucose-dependent inhibition of glucagon secretion, and improved β-cell function.1 As the insulinotropic effect of GLP-1 is preserved in type 2 diabetes mellitus (T2DM), it has created considerable interest as an agent for the treatment of T2DM. However, since endogenous GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4), resulting in a short half-life in humans (26 minutes) after intravenous (IV) administration,2 the therapeutic use of endogenous GLP-1 is limited. Instead, several derivatives with a longer duration of action have been developed.3,4 Liraglutide is a GLP-1 analog that shares 97% amino acid sequence identity with native GLP-1.5 The analog is obtained by creating two modifications to native GLP-1: the replacement of lysine with arginine at position 34 and the attachment of a C16 fatty acid chain via a γ-glutamic acid spacer to lysine at position 26.5 The fatty acid chain contributes to delaying absorption and extending the plasma half-life by increased binding to plasma albumin, while the lysine replacement makes DPP-4 unable to exert its action. Clinical trials have shown that liraglutide significantly reduces fasting plasma glucose and HbA1c and does so with a low risk of hypoglycemia in T2DM.6

The aim of this work was to evaluate the effects of a GLP-1 analog on glucose homeostasis in T2DM patients by use of the previously developed integrated glucose-insulin (IGI) model7–10 following appropriate modification. The model was developed to describe the glucose homeostasis in nondiabetic subjects and T2DM patients during different glucose provocations, including intravenous glucose tolerance tests (IVGTTs), oral glucose tolerance tests (OGTTs), and meal tolerance tests (MTTs).7–10 The model has also been used to describe glucose homeostasis in T2DM patients treated with different antidiabetic drugs, such as a glucokinase activator11 and an insulin analog.12 By including drug effects in the model, it becomes a useful tool in drug development, as it may aid the selection of dosing regimen for phase II trials.13 Thus, by extending the model with a component for liraglutide, as an example of a GLP-1 analog, it becomes useful in investigating new analogs and their effects on glucose homeostasis.

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Pharmacokinetics of liraglutide

The PK was described by a one-compartment model with first-order absorption and first-order elimination parameterized as absorption rate constant ($k_{aL}$), clearance ($CL_{L}/F$), and volume of distribution ($V_{L}/F$) and with between-subject variability parameters on clearance and volume. Another PK model for liraglutide was also tested. In this model liraglutide absorption is described by a dose-proportional zero-order process and a subsequent first-order process. However, this model did not improve the model fit judged by assessment of individual profiles, objective function value, and goodness of fit plots. Thus, it was decided to keep the simpler model with first-order absorption. In the final model clearance and volume of distribution were assumed to be proportional to weight. The final parameter estimates are given in Table 1. A bootstrap with 100 samples was performed to compare the standard errors (SEs) from the bootstrap with those estimated by NONMEM. The agreement between the SE estimates was good (Table 1). The PK model was able to describe the data well, as seen in the left panel of Figure 2, and showed reasonable predictive performance, as illustrated in a visual predictive check (VPC) in the right panel of Figure 2. The VPC, which is based on 1,000 simulations, shows that the main trend of the data is well captured by the model. However, the variability appears to be overestimated. In the figures it appears as though there is a plateau near the $C_{\text{max}}$. This plateau appears because data from the two trials are pooled, and in trial 1 there are only four samples per subject which are all near $C_{\text{max}}$. If data from trial 1 are omitted, no plateau is seen.

Pharmacodynamics of glucose and insulin

As liraglutide is known to stimulate insulin secretion in a glucose-dependent manner, the expression for insulin secretion in the IGI model was altered. In the IGI model the insulin secretion ($I_{\text{sec}}$) is governed by the product of three contributions: the basal production, the glucose-stimulated production, and the endogenous incretin effect (model equations are provided in the Supplementary Material). The effect of liraglutide was incorporated by multiplying $I_{\text{sec}}$ with a stimulatory function $F(C_{\text{lira}})$, where $C_{\text{lira}}$ is the concentration of liraglutide. The function had to satisfy the conditions that $F(C_{\text{lira}})=1$ for $C_{\text{lira}}=0$ and $F(C_{\text{lira}})\geq 1$ for $C_{\text{lira}}\geq 0$. Different expressions for $F(C_{\text{lira}})$ were tested (linear and Emax), and in the final model a linear function was used:

$$F(C_{\text{lira}})=1+p_{\text{lira}} C_{\text{lira}}$$

$C_{\text{lira}}$ was predicted for each individual subject using the PK model for liraglutide with fixed population parameters. The parameter $p_{\text{lira}}$ is an estimated constant describing the linear slope between $C_{\text{lira}}$ and the effect on insulin secretion. This model was chosen due to its simplicity while still adequately describing the data.

In both trials, 1.5 g of paracetamol was administered with the meal to assess the gastric emptying rate. Paracetamol data from trial 1 indicated a delay in gastric emptying with liraglutide during the early phase after the meal (data not shown). No delay in gastric emptying was observed in trial 2. Assuming glucose is not absorbed in the stomach but in the intestine, the glucose absorption rate, $k_{a}$, reflects the gastric emptying rate. A significant model improvement was obtained by allowing $k_{a}$ to be reduced in trial 1 for the MTTs with liraglutide, such that two values for $k_{a}$ were estimated: one for the placebo data ($k_{a,\text{trial1 placebo}}$) and one for the liraglutide data ($k_{a,\text{trial1 liraglutide}}$). It was not possible to detect any liraglutide concentration-dependent reduction in $k_{a}$. No model improvement was achieved by including different values for $k_{a}$ for the placebo and the liraglutide arm in trial 2.

In trial 2 the glucagon response was significantly reduced with liraglutide vs. placebo (no glucagon measurements were obtained in trial 1). Glucagon is not explicitly expressed in the IGI model; however, the IGI model for healthy subjects contains a delayed, glucose-dependent inhibition of endogenous glucose production (EGP), which accounts for the effect of glucagon. As T2DM patients have been shown to have a malfunction in the suppression of EGP, the IGI model for T2DM patients does not contain this feedback mechanism. However, in another trial the rate of EGP in the fasting state was significantly reduced in liraglutide-treated T2DM patients. Thus, it seems reasonable to assume that liraglutide has an effect on EGP. This was tested by including an inhibitory effect of $C_{\text{lira}}$ on EGP. Different inhibitory functions were tested (linear and Emax), but they did not improve the fit nor were they statistically significant. For each inhibitory function it was checked that, with the estimated parameters, EGP remained positive at all relevant conditions.
liraglutide concentrations. No effect of liraglutide on EGP was included in the final model.

The IGI model was able to describe the glucose and insulin data well, as seen in Figures 3 and 4, in which time courses for plasma glucose and insulin concentrations for the two trials are shown together with the model fits. The MTTs with liraglutide were performed when liraglutide was at steady state. Thus, the glucose concentration at the start of the MTTs with liraglutide was less than the estimated glucose concentration at steady state $G_{SS}$, which reflects the placebo steady-state concentration. In the model, all previous liraglutide doses are included to ensure that at the start of the MTTs with liraglutide the glucose concentration was less than $G_{SS}$. For example, for trial 1 the estimate of $G_{SS}$ was 9.7 mM. At the start of the MTT with 0.6 mg liraglutide, the mean of the observed glucose concentrations was 8.1 mM, which is in accordance with the mean of the individual model predictions of 8.5 mM. At the start of the MTT with 1.2 mg liraglutide the mean observed glucose concentration was 7.6 mM and the mean of the individual

| Description                        | Typical value | RSE, % |
|------------------------------------|---------------|--------|
| Liraglutide                         |               |        |
| $V_L/F$ [L/kg]                      | 0.112         | 14 (14) |
| $CL_L/F$ [L/min/kg]                 | 1.83 $10^{-4}$| 10 (10) |
| $k_a$ [1/min]                       | 9.41 $10^{-4}$| 9 (9)  |
| IIV $V_L$, %                        | 48            | 21 (21) |
| IIV $CL_L$, %                       | 52            | 29 (29) |
| RESL, %                             | 14            | 6 (7)   |
| Glucose                             |               |        |
| $CL_G$ [L/min]                      | 0.0287        | —      |
| $CL_{GI}$ [L/min/(pM)]              | 9.6 $10^{-4}$ | 13     |
| $V_P$ [L]                           | 8.56          | —      |
| $V_C$ [L]                           | 9.33          | —      |
| $Q$ [L/min]                         | 0.442         | —      |
| $k_{DEG}$ [1/min]                   | 0.0289        | —      |
| $k_a$, trial 1, placebo [1/min]     | 0.0278        | 7      |
| $k_a$, trial 1, liraglutide [1/min] | 0.0151        | 8      |
| $k_a$, trial 2 [1/min]              | 0.0143        | 10     |
| $D_{trial 1}$ [g]                   | 51            | 7      |
| $D_{trial 2}$ [g]                   | 82            | 11     |
| $G_{SS}$, trial 1 [mM]              | 9.67          | 4      |
| $G_{SS}$, trial 2 [mM]              | 7.34          | 5      |
| IIV $CL_G$, %                       | 59            | —      |
| IIV $CL_{GI}$, %                    | 53            | 18     |
| IIV $CL_I$, %                       | 29            | —      |
| Insulin                             |               |        |
| $I_{SS}$, trial 1 [pM]              | 36.9          | 12     |
| $I_{SS}$, trial 2 [pM]              | 41.5          | 13     |
| $V_I$ [L]                           | 6.09          | —      |
| $CL_I$ [L/min]                      | 1.22          | —      |
| $k_{IE}$ [1/min]                    | 0.0213        | —      |
| $IPRG$                              | 1.42          | —      |
| $E_{max}$                           | 13.9          | 25     |
| $ED_{50}$ [g]                       | 108           | 29     |
| $P_{max}$ [nM]                      | 0.0665        | 20     |
| Residual error                      |               |        |
| RESG, %                             | 16            | 6      |
| RESI, %                             | 64            | 13     |

For the liraglutide model SE estimates were obtained both from the NONMEM run and by a bootstrap with 100 samples, the estimates from the bootstrap are in parentheses. For the IGI model all SEs were obtained from a bootstrap with 50 samples. IIV%, interindividual variability as standard deviation in percentage. Estimated parameters are in bold, all other parameters are fixed to the values from Jauslin et al. $V_C$ and $V_P$ are proportional to weight, which is incorporated as a covariate and normalized to 70 kg. $V_I$ and $CL_I$ are proportional to weight. RSE%, relative standard error in percentage.
model predictions was 7.7 mM. At the start of the MTT with 1.8 mg liraglutide the mean observed glucose concentration was 7.3 mM and the mean of the individual model predictions was 7.1 mM. There seems to be a trend in the insulin concentrations that the model does not quite capture (underprediction at the low liraglutide doses and overprediction at the higher liraglutide doses).

The effect of liraglutide on insulin secretion is illustrated in Figure 5, in terms of model predicted insulin secretion for trial 1 at all dose levels (left panel). The right panel of Figure 5 shows the mean baseline corrected postprandial plasma glucose at all dose levels, for comparison. As seen, liraglutide exhibited a dose-dependent stimulation of insulin secretion concurrently with a dose-dependent reduction in glucose excursions following meal intake.

The predictive performance of the model was graphically evaluated by a VPC based on 1,000 simulations. The VPC is included in the Supplementary Material. The VPC shows that the main trend and the variability of glucose and insulin were well captured by the model at all dose levels. As the final model terminated with rounding errors, standard errors in the IGI model were obtained by a bootstrap. Due to long run times (~18 hours), the number of bootstrap samples was limited to 50, which was deemed sufficient for the estimation of standard errors.

**DISCUSSION**

In recent years, several GLP-1-based therapies have been developed. These therapies can be divided into two groups:

(i) dipeptidyl-peptidase 4 inhibitors, such as vildagliptin and sitagliptin, which increase the concentration of native GLP-1, and (ii) GLP-1 receptor agonists, such as liraglutide and exenatide.

Different models have been used to describe the effect of GLP-1-based therapies on glucose homeostasis. A mechanism-based population model of the effects of vildagliptin on GLP-1, glucose, and insulin in T2DM patients was developed by Landersdorfer et al.\(^{19}\) The effect of exenatide on insulin secretion during a hyperglycemic clamp\(^{20}\) as well as the effect of liraglutide on glucose and insulin homeostasis in healthy volunteers\(^{21}\) have been explored using an adapted minimal model. The effects of liraglutide in the adapted minimal model were implemented as a stimulatory effect on the magnitude of the first-phase insulin secretion and an increased second-phase insulin secretion. Also, exenatide was found to increase second-phase insulin secretion.

In this study the integrated glucose-insulin model was extended to describe the glucose homeostasis in T2DM patients treated with liraglutide. For this purpose data from two trials investigating the effect of steady-state liraglutide on glucose homeostasis were used.

The main effect of liraglutide on glucose homeostasis was incorporated in the IGI model as a concentration-dependent stimulation of insulin secretion. This is in accordance with prior modeling of the effects of GLP-1 analogs.\(^{20,21}\) No significant model improvement was achieved by including an inhibitory effect of liraglutide on the endogenous glucose production, which would mimic
Figure 3 Time courses for plasma glucose and insulin concentrations for trial 1. The dots show the geometric mean and the corresponding 95% confidence interval of the data. The solid lines show the geometric mean of the individual predictions (solid lines) and the geometric mean of the population predictions (dashed lines). Time zero is defined as the time of the first measurement in the MTT, which is 10 minutes before meal ingestion.
an effect on glucagon. Furthermore, in one of the two trials (trial 1) a significant model improvement was achieved by including an inhibitory effect of liraglutide on gastric emptying. As it was assumed that glucose is absorbed in the intestine and not in the stomach, this was modeled as a decreased glucose absorption rate for the MTTs with liraglutide.

The estimated glucose absorption rate in trial 1 was nearly twice as high as in trial 2. The caloric content of a meal is known to affect the gastric emptying rate, with decreased gastric emptying rate for increased caloric density. In trial 2 the caloric content of the meals was twice as large as in trial 1. Thus, it was reasonable to assume that the gastric emptying rate in trial 2 was smaller than in...
In this article, data from trials with liraglutide were used for model development. However, as other GLP-1 receptor agonists have similar modes of action as liraglutide, it is believed that the model with minor alterations can also be used to describe glucose homeostasis in T2DM patients treated with other GLP-1 receptor agonists. Others are also working on implementing the effects of GLP-1 receptor agonists on glucose homeostasis in the IGI model, and have presented similar results. Thus, we conclude that the IGI model is a useful tool for investigating GLP-1 receptor agonists.

METHODS

Data

For model development data from the following two trials were used.

Trial 1

Trial 1 was a single-center, randomized, placebo-controlled, double-blind, two-period, crossover trial, comparing the effect of steady-state liraglutide at three dose levels (0.6, 1.2, and 1.8 mg/day) vs. placebo on the responses of fasting plasma glucose and postprandial glucose, insulin, and gastric emptying in T2DM patients. Eighteen patients were recruited for the trial and they were randomly assigned to either treatment group A (3 weeks of once-daily liraglutide, 3–4 weeks of washout, and 3 weeks of once-daily placebo) or B (3 weeks of once-daily placebo, 3–4 weeks of washout, and 3 weeks of once-daily liraglutide). During each 3-week treatment period, the liraglutide/placebo dose was escalated weekly in 0.6-mg increments from 0.6 mg until a daily dose of 1.8 mg was reached. After 1 week of treatment at each dose level, an energy fixed MTT was performed. The patients were instructed to administer liraglutide/placebo in the evening and the MTT was performed in the morning. Along with the meal, patients also received 1.5 g paracetamol. During the MTT, plasma glucose, serum insulin, and paracetamol concentrations were measured. A baseline sample was drawn 10 minutes before the MTT and during the postprandial period nine blood samples were drawn at the following timepoints: 15, 30, 45, 60, 90, 120, 180, 240, and 300 minutes. Blood samples for assessment of liraglutide concentrations were drawn 6, 8, 10, and 12 hours after the liraglutide/placebo dosing. For more information on the trial, the reader is referred to Flint et al.14

Trial 2

Trial 2 was a randomized, double-blind, placebo-controlled, crossover trial, comparing the effect of treatment with steady-state liraglutide 1.8 mg vs. placebo on postprandial plasma triglyceride concentrations following a standardized high-fat meal in T2DM patients. Treatment order of liraglutide 1.8 mg or placebo was determined through random assignment. Individuals randomized to liraglutide underwent weekly dose escalation, starting at 0.6 mg/day (with 0.6-mg increments to 1.8 mg). Liraglutide vehicle was used as placebo to ensure blinding. A washout period of 3–9 weeks was included between the two treatments. At the end of each 3-week period an MTT was performed. The patients

Figure 5 (Left) Model predicted insulin secretion for the different dose levels for trial 1. (Right) Mean baseline corrected postprandial plasma glucose profiles during the MTTs for trial 1. All placebo data was included in the calculation of the mean, i.e., each subject contributes with three placebo profiles.
were instructed to administer liraglutide/placebo in the evening and the MTT was performed in the morning. Along with the meal, patients also received 1.5 g paracetamol. During the MTT, plasma glucose, serum insulin, and paracetamol concentrations were measured. A baseline sample was drawn 15 minutes before the MTT and during the postprandial period nine blood samples for glucose and insulin were drawn at the following timepoints: 15, 30, 60, 120, 180, 240, 300, 360, and 480 minutes. Blood samples for assessment of liraglutide concentrations were drawn at 15 minutes before and 4, 6, 8, 10, 12, 14, 16, 24, 36, 48, and 60 hours after the last liraglutide dose.

Eighteen patients completed the trial. However, due to noncompliance with protocol requirements, meal-test endpoints for seven individuals were excluded from the analysis. For additional information on the trial, the reader is referred to Hermansen et al.15

**Model development**

The integrated glucose-insulin (IGI) model developed by Silber et al.9 was used as a basis for model development. The model consists of submodels for glucose and insulin as well as homeostatic feedback mechanisms which control the insulin secretion and the elimination of glucose (the model equations are provided in the Supplementary Material). To account for the effect of liraglutide on glucose homeostasis, a PK model for liraglutide was coupled to the IGI model (Figure 1). The effect of liraglutide on glucose homeostasis was tested on relevant parts of the model (insulin secretion, endogenous glucose production, and glucose absorption rate).

**Glucose submodel**

Glucose was described by a two-compartment disposition model with both insulin-dependent and insulin-independent elimination from the central compartment and endogenous glucose production. The glucose absorption from the gut was described by a first-order process via one transit compartment. The stimulatory effect of glucose on insulin secretion was included in the model using an effect compartment.

**Endogenous insulin submodel**

Endogenous insulin kinetics were described by a one-compartment model with endogenous insulin secretion and first-order elimination. The endogenous insulin secretion was assumed to be regulated by the blood glucose concentration and the incretin effect. The incretin effect was described by a saturable function linking the amount of glucose in the glucose transit compartment to insulin secretion.

**Model estimation and statistical analysis**

Model estimation was performed using nonlinear mixed effects modeling in NONMEM 727 with the first-order conditional estimation (FOCE) method and the differential equation solver ADVAN13. The NONMEM code for the final model is provided in the Supplementary Material. Model selection was based on a compromise between mechanistic considerations, objective function value (OFV), plausibility of parameter estimates, and graphical assessment. A difference in the OFV of at least 10.83 was used as a cutpoint value for nested models differing by one parameter, which corresponds to a significance level of α = 0.001.

**Interindividual variability.** The differences between individual parameters were described using random effects, which were assumed to be normally distributed with mean zero. The distribution of the individual parameters around the typical population value was assumed to be log-normal.

**Residual error model.** An additive model was used to describe the residual error of the log-transformed data. The residual error was assumed to arise from a distribution with mean zero and an estimated variance. Different residual errors for glucose (RESG), insulin (RESI), and liraglutide (RESL) were estimated.

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**Conflict of Interest.** R.M.R., S.K., S.H.I., and N.R.K. are employed at Novo Nordisk A/S.

**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?**

✓ The integrated glucose-insulin model, which was previously developed to describe the glucose homeostasis during different glucose provocations, has been used to investigate the effects of antidiabetic drugs on glucose homeostasis.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

✓ Can the integrated glucose-insulin model be extended to describe glucose homeostasis in T2DM patients treated with the GLP-1 receptor agonist liraglutide at different dose levels?

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

✓ Effects of liraglutide on glucose homeostasis were included in the model, which was found suitable for clinical trial simulations.
As other GLP-1 receptor agonists have similar modes of action as liraglutide, the model may potentially be used to describe the glucose homeostasis in T2DM patients treated with other GLP-1 receptor agonists or possibly combination products.