

## ARTICLE

# A Physiologically-Based Quantitative Systems Pharmacology Model of the Incretin Hormones GLP-1 and GIP and the DPP4 Inhibitor Sitagliptin

Pavel Balazki<sup>1,2,3</sup>, Stephan Schaller<sup>3</sup>, Thomas Eissing<sup>2</sup> and Thorsten Lehr<sup>1,\*</sup>

Incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) play a major role in regulation of postprandial glucose and the development of type 2 diabetes mellitus. The incretins are rapidly metabolized, primarily by the enzyme dipeptidyl-peptidase 4 (DPP4), and the neutral endopeptidase (NEP), although the exact metabolization pathways are unknown. We developed a physiologically-based (PB) quantitative systems pharmacology model of GLP-1 and GIP and their metabolites that describes the secretion of the incretins in response to intraduodenal glucose infusions and their degradation by DPP4 and NEP. The model describes the observed data and suggests that NEP significantly contributes to the metabolization of GLP-1, and the traditional assays for the total GLP-1 and GIP forms measure yet unknown entities produced by NEP. We further extended the model with a PB pharmacokinetics/pharmacodynamics model of the DPP4 inhibitor sitagliptin that allows predictions of the effects of this medication class on incretin concentrations.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are rapidly metabolized by dipeptidyl-peptidase 4 (DPP4), but recent data suggest the presence of further metabolization processes. No mathematical models of incretins with mechanistic description of the processes exist.

### WHAT QUESTION DID THIS STUDY ADDRESS?

✓ Development of a mathematical model of secretion and metabolization of GLP-1 and GIP and a DPP4 inhibitor based on the current knowledge of the underlying mechanisms for integration into computational diabetes platforms.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✓ The model allows predictions of the unknown GLP-1 metabolite and the effect of DPP4 inhibitors based on

their mode of action. Analysis of incretins data across different sources stresses the need in standardization in GLP-1 and GIP measurements to assess the interindividual variability.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

✓ The study reveals the importance of pathways other than DPP4-mediated degradation in metabolization of the incretins. New treatments targeting the new pathway could be developed. The mechanism-based model allows *in silico* testing of such new therapies and predictions of personalized treatment outcomes for medication combinations.

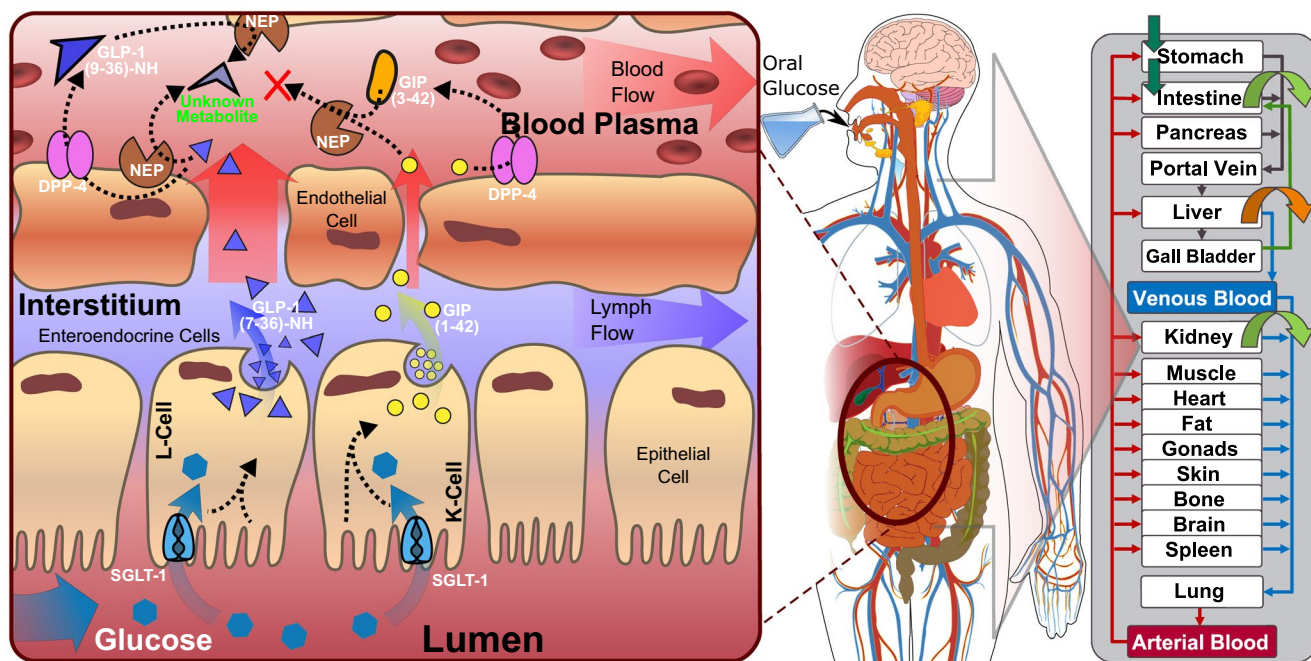
The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are crucial for the regulation of postprandial glucose levels. The peptides are secreted from endocrine cells located in the gut mucosa as a response to meal ingestion<sup>1</sup> and contribute to controlling blood glucose levels by potentiating insulin secretion (GLP-1 and GIP) and decreasing the gastric emptying rate (GLP-1).

Secreted GLP-1 and GIP are subject to rapid metabolization by the enzyme dipeptidyl peptidase 4 (DPP4), which is expressed on the membranes of various tissues, including

the endothelial cells<sup>2</sup> and can be found in soluble form in blood plasma.<sup>3</sup> The enzyme converts the biologically active forms GLP-1 (7-36)amide to GLP-1 (9-36)amide and GIP (1-42) to GIP (3-42) (**Figure 1**) with estimated half-life values of 2 and 7 minutes, respectively.<sup>1,4</sup> *In vitro* studies show that GLP-1 and, to a smaller extent, GIP can also be metabolized by the enzyme neutral endopeptidase (NEP) 24.11.<sup>5</sup> Although the contribution of NEP to the degradation of active GLP-1 *in vivo* has been considered negligible,<sup>4</sup> the primary metabolite GLP-1 (9-36)amide is extensively eliminated by NEP in pigs<sup>6</sup> and mice.<sup>7</sup>

<sup>1</sup>Clinical Pharmacy Department, Saarland University, Saarbrücken, Germany; <sup>2</sup>Clinical Pharmacometrics Department, Bayer AG, Leverkusen, Germany; <sup>3</sup>esqLABS GmbH, Saterland, Germany. \*Correspondence: Thorsten Lehr ([thorsten.lehr@mx.uni-saarland.de](mailto:thorsten.lehr@mx.uni-saarland.de))

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**Figure 1** Physiology of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) pharmacokinetic as modeled. GLP-1 and GIP are produced in the L-cells and K-cells of intestinal mucosa, respectively. The uptake of glucose from the gut lumen by the sodium/glucose cotransporter 1 (SGLT1) transporters enhance the secretion of the incretins. After secretion into interstitial space, the hormones are transported through the whole body by plasma and lymph flows. GLP-1 (7-36)amide is metabolized by dipeptidyl-peptidase 4 (DPP4; located in plasma and endothelial membrane) to GLP-1 (9-36)amide and by neutral endopeptidase (NEP; located in endothelial membrane) to an unknown metabolite. GLP-1 (9-36)amide is also metabolized by NEP to the unknown metabolite, which is removed renally. GIP (1-42) is metabolized by DPP4 to GIP (3-42)amide. Additionally, GIP (1-42)amide and (3-42)amide are removed by NEP. Assays measuring total GLP-1 concentration are assumed to measure the sum of GLP-1 (7-36)amide, GLP-1 (9-36)amide, and the unknown metabolite. Assays measuring the total GIP concentration are assumed to measure the sum of GIP (1-42)amide and GIP (3-42)amide.

Due to its importance in glucose homeostasis, the incretin system is a promising target for the treatment of type 2 diabetes mellitus (T2DM). The common strategy is to either increase the concentrations of active endogenous GLP-1 by inhibition of DPP4,<sup>8</sup> or to design GLP-1 analogues that are protected from degradation by DPP4.<sup>9</sup> GIP was not considered a potent drug target for the treatment of T2DM for a long time. However, the interest in GIP may increase, as recently a dual GLP-1 and GIP receptor agonist has demonstrated superior glycemic control compared with pure GLP-1 agonists.<sup>10</sup>

Concentrations of intact GLP-1 after administration of DPP4 inhibitors (DPP4is) increase by less than twofold.<sup>11,12</sup> The understanding of this relatively small effect of DPP4i depends on correct assumptions regarding the mechanisms of incretin's metabolization. However, the high heterogeneity and poor comparability of reported incretin concentration data does not allow to clearly define the processes involved in the degradation of GLP-1 and GIP. Most of the investigators do not distinguish between the intact forms of the peptides and their primary metabolites and report "total" concentrations, which are assumed to be the sum of the active forms and the DPP4-produced metabolites. This assumption is challenged by the work of Albrechtsen *et al.*<sup>13</sup> who developed an assay specific for GLP-1 (9-36)amide. The assay revealed that the sum of GLP-1 (7-36)amide and (9-36)amide concentrations is lower than the measured total

concentration,<sup>13</sup> suggesting the presence of an additional form of GLP-1 detected by the "total" assays and a possibly bigger role of NEP in metabolization of the incretins.

As for today, no mathematical models exist that would allow to quantitatively describe and predict the dynamics of the different forms of the incretins. In this work, we present a physiologically-based (PB)<sup>14</sup> quantitative systems pharmacology model of GLP-1 and GIP metabolism and secretion to (i) assess and investigate the sources of variability in reported incretin concentrations, to (ii) provide a reliable model of endogenous incretins for the integration in digital diabetes disease platforms, and (iii) to develop and integrate a PB pharmacokinetic (PK) model of the DPP4i sitagliptin and its pharmacodynamic (PD) mechanisms to predict the effects of DPP4i therapy.

## METHODS

The presented open source ordinary differential equations based quantitative systems pharmacology model was developed with PK-Sim and MoBi as part of the Open Systems Pharmacology Suite, version 8.<sup>15</sup> Figures were created in R<sup>16</sup> with the Open Systems Pharmacology Suite Toolbox for R (version 8).

The PBPK models were created in PK-Sim and coupled in MoBi. Unknown parameter values were estimated by fitting the model to experimental data with the parameter

identification tool in MoBi, applying a combination of the Levenberg–Marquardt and Monte–Carlo algorithms.

Parameters describing metabolism of GLP-1 and GIP were estimated by fitting the model to one data set reporting the concentrations of GLP-1 (and its metabolites) and one data set reporting the concentrations of GIP, respectively. The other data sets listed below were used for model qualification.

Data sets used for the definition and validation of GLP-1 metabolism processes are listed in **Table S1** and include concentration-time measurements during i.v. infusions of GLP-1 with infusion rates ranging between 0.5 and 2 pmol/kg body weight (BW)/min. In total, 24 GLP-1 concentration data sets were extracted. Six sources were excluded from the comparison as the reported dose-normalized concentrations markedly differed from the values reported in other sources; these are marked as “outlier” in **Table S1**.

Data sets used for the definition and validation GIP metabolism processes are listed in **Table S2**. The seven data sets include concentration-time profiles of GIP during i.v. infusions with infusion rates ranging between 0.8 and 16 pmol/kg BW/min.

Data sets used for the definition and validation of the secretion processes are listed in **Table S3**. The data sets include concentration-time profiles of GLP-1 and GIP during intraduodenal (i.d.) infusions of glucose at rates ranging from 0.71 to 4 kcal/min. The choice of i.d. infusion protocols rather than oral administrations of glucose allows to exclude the influence of gastric emptying and to assess the impact of intestinal lumen exposure to glucose in a controlled setting.

Some sources report concentrations of GLP-1 and GIP sampled from dorsal hand veins by applying the “heated-hand” technique. Applying heat to the hand allows sampling of blood that is more similar to the arterial blood—the so-called arterialized venous blood (AVB).<sup>17</sup> AVB is modeled as a mixture of the standard peripheral venous blood as defined by PK-Sim, and arterial blood. Detailed description of the calculation of AVB concentrations is provided in **Supplementary Text S1**.

The PBPK model of sitagliptin was developed with data sets listed in **Table S4**. In total, three concentration-time profiles gathered after i.v. administration of sitagliptin (25, 50, and 100 mg doses) and 21 profiles after oral (p.o.) administrations (dose range 1.5–800 mg) were used. The parameters describing the PD effect of sitagliptin on inhibition of DPP4 were estimated with data sets describing DPP4 activity in plasma after p.o. administration in the dose range between 1.5 and 200 mg.

All simulations discussed in this work can be found in the MoBi project file provided in the **Supplementary Material**, the results are depicted in **Figures S1–S26**.

## RESULTS

The developed model describes the secretion of GLP-1 (7-36)amide and GIP (1-42)amide and the metabolism of the secreted forms and their metabolites GLP-1 (9-36)amide, the unknown GLP-1 metabolite produced by NEP, and GIP

(3-42)amide. The implemented processes are outlined in **Figure 1**.

Detailed descriptions of the GLP-1 and GIP metabolism models are provided in **Supplementary Text S3 and S4**, the secretion processes are described in **Supplementary Text S5**. The PBPK/PD model of the DPP4i sitagliptin is described in **Supplementary Text S6**. Parameters of the incretin models are listed in **Tables S5–S7**, parameters describing the sitagliptin model are listed in **Table S8**.

### GLP-1 PBPK model

GLP-1 (7-36)amide is secreted from the L-cells located in the intestinal mucosa<sup>4</sup> and is converted to GLP-1 (9-36)amide by DPP4. The DPP4 is modeled in endothelial cell membrane and free floating in plasma. Reported values for plasma DPP4 concentrations vary between 23.5 and 5,690 ng/mL,<sup>18–21</sup> and the median value of 913 ng/mL with the molecular weight of DPP4 of 175 kDa<sup>22</sup> is used in the model.

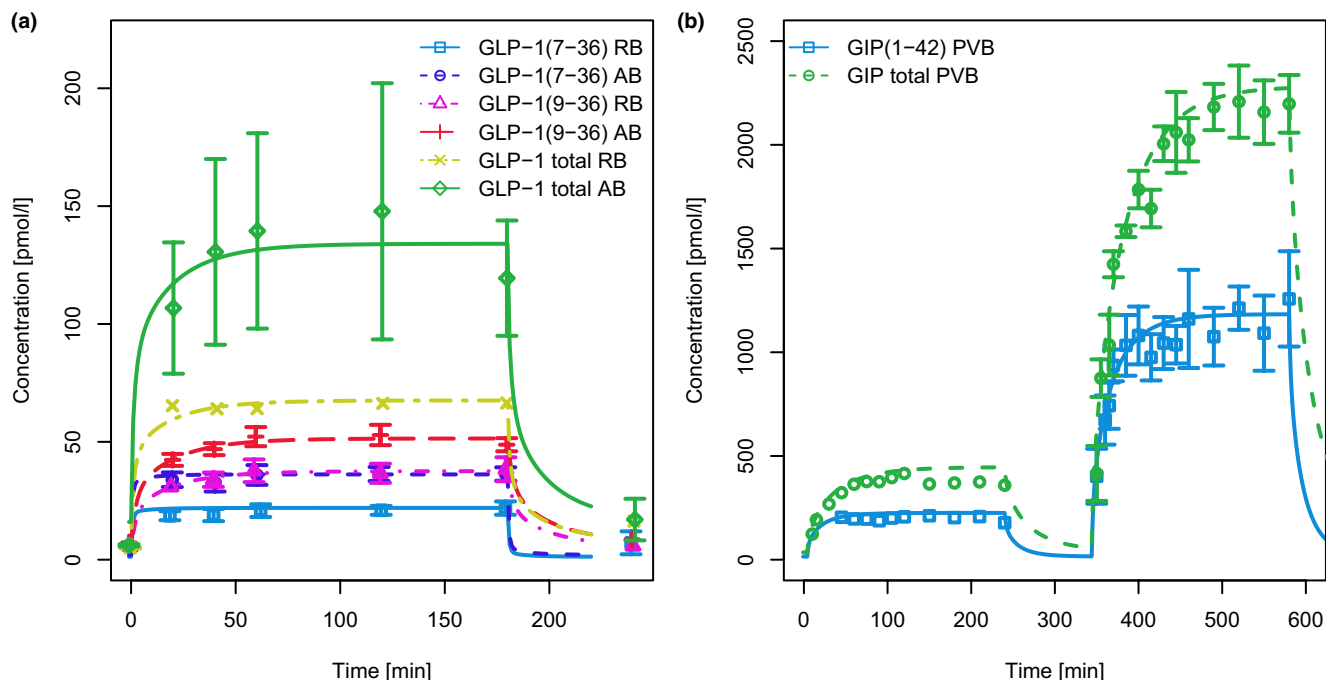
As the data by Albrechtsen *et al.*<sup>13</sup> indicates the presence of GLP-1 fragments other than GLP-1 (9-37)amide, degradation of GLP-1 (7-36)amide and GLP-1 (9-36)amide by NEP to an unknown metabolite “GLP-1\_NEP\_Metabolite” is included. The total concentration of GLP-1 is defined as the sum of GLP-1 (7-36)amide, GLP-1 (9-36)amide, and GLP-1\_NEP\_Metabolite concentrations. GLP-1\_NEP\_Metabolite is removed through a further degradation by NEP, the product of which is not measured by any GLP-1 assay. All forms are cleared by the kidneys through glomerular filtration and tubular secretion.

The parameter values were estimated by fitting the model to concentration-time profiles reported by Albrechtsen *et al.*<sup>13</sup> for the healthy group. The model accurately describes the concentrations of GLP-1 (7-36)amide, GLP-1 (9-36)amide, and the total GLP-1 in arterial and renal blood plasma during an infusion of 1.5 pmol/kg BW/min of GLP-1 (**Figure 2a**).

The predictions of active GLP-1 concentrations for 8 data sets (see **Table S1**) are in good accordance with the observed values, with 11 of 75 compared points (15%) being outside of the twofold difference range (**Figure 3a**). The highest fold-differences between the observed and the predicted values are observed at basal concentrations of the hormone, with reported values ranging from  $< 1^{11}$  to  $15^{23}$  pmol/L. The data sets that used the Millipore ELISA kit for determining active GLP-1 concentrations<sup>24,25</sup> reported approximately three-fold to seven-fold higher concentrations than those simulated by the model and are considered as outliers (**Figures S17 and S18**). The four other outlier data sets used the assays that were also applied in correctly predicted data sets, and no further assay-dependent bias was observed.

The predictions of total GLP-1 concentrations are compared with observed values in **Figure 3b**. Of the 242 compared points from 19 data sets, 29 (12%) are outside of the twofold difference range. No systemic bias was observed for any of the total GLP-1 assays.

The underpredictions or overpredictions of active and total GLP-1 do not correlate. A simulation of 1.2 pmol/kg BW/min GLP-1 infusion reported by Toft-Nielsen *et al.*<sup>26</sup> resulted in perfect prediction of the total GLP-1 levels,



**Figure 2** Performance of the glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) physiologically-based pharmacokinetic models. Comparison of simulated (lines) and observed (symbols) concentrations of incretins. (a) GLP-1 (7-36) amide, GLP-1 (9-36)amide, and total GLP-1 in arterial (AB) and renal blood (RB) during 180-minute infusion of 1.5 pmol/kg body weight (BW)/min GLP-1. Data from Albrechtsen *et al.*<sup>13</sup> (b) GIP (1-42)amide and total GIP in peripheral venous blood (PVB) during 4 (minutes 0–240) and 16 (minutes 340–580) pmol/kg BW/min GIP infusions. Data from Vilsbøll *et al.*<sup>27</sup>

but a 34% underprediction of the active concentrations (**Figure S14**). Simulation of 1 pmol/kg BW/min reported by Vilsbøll *et al.*<sup>27</sup> resulted in very good prediction of active GLP-1, but the total concentrations were underpredicted by ~ 30 pmol/L (25%; **Figure S16**). Both sources used the same assays for determining active and total GLP-1 concentrations and the demographic characteristics of subjects in the studies were similar.

#### GIP PBPK model

GIP (1-42)amide is secreted by the K-cells<sup>4</sup> and is converted to GIP (3-42)amide by DPP4. Both GIP (1-42)amide and (3-42)amide are degraded by NEP. All forms are cleared by the kidneys through glomerular filtration. The total concentration of GIP is defined as the sum of GIP (1-42)amide and (3-42)amide. Parameter values were estimated by fitting the model to concentration-time profiles obtained during i.v. infusions of 4 and 16 pmol/kg BW/min GIP reported by Vilsbøll *et al.*<sup>27</sup>

The comparison of fitted and observed GIP (active and total) concentrations is presented in **Figure 2b**. The predictive performance was qualified by simulating reported i.v. infusion experiments (**Table S2**). The only data set that reports the concentrations of active GIP during an infusion<sup>28</sup> that has not been used for parameters estimation is overpredicted by 70% (**Figure 3c** and **Figure S19**). The predictions of total GIP concentrations were good for two data sets (**Figures S20–S21**),<sup>25,29</sup> two other data sets were underpredicted<sup>30</sup> and overpredicted<sup>31</sup> by approximately twofold (**Figures S22–S23**), and another data set<sup>32</sup> was underpredicted by approximately fourfold (**Figure S24**). Collected

comparison of predicted vs. observed values is presented in **Figure 3d**.

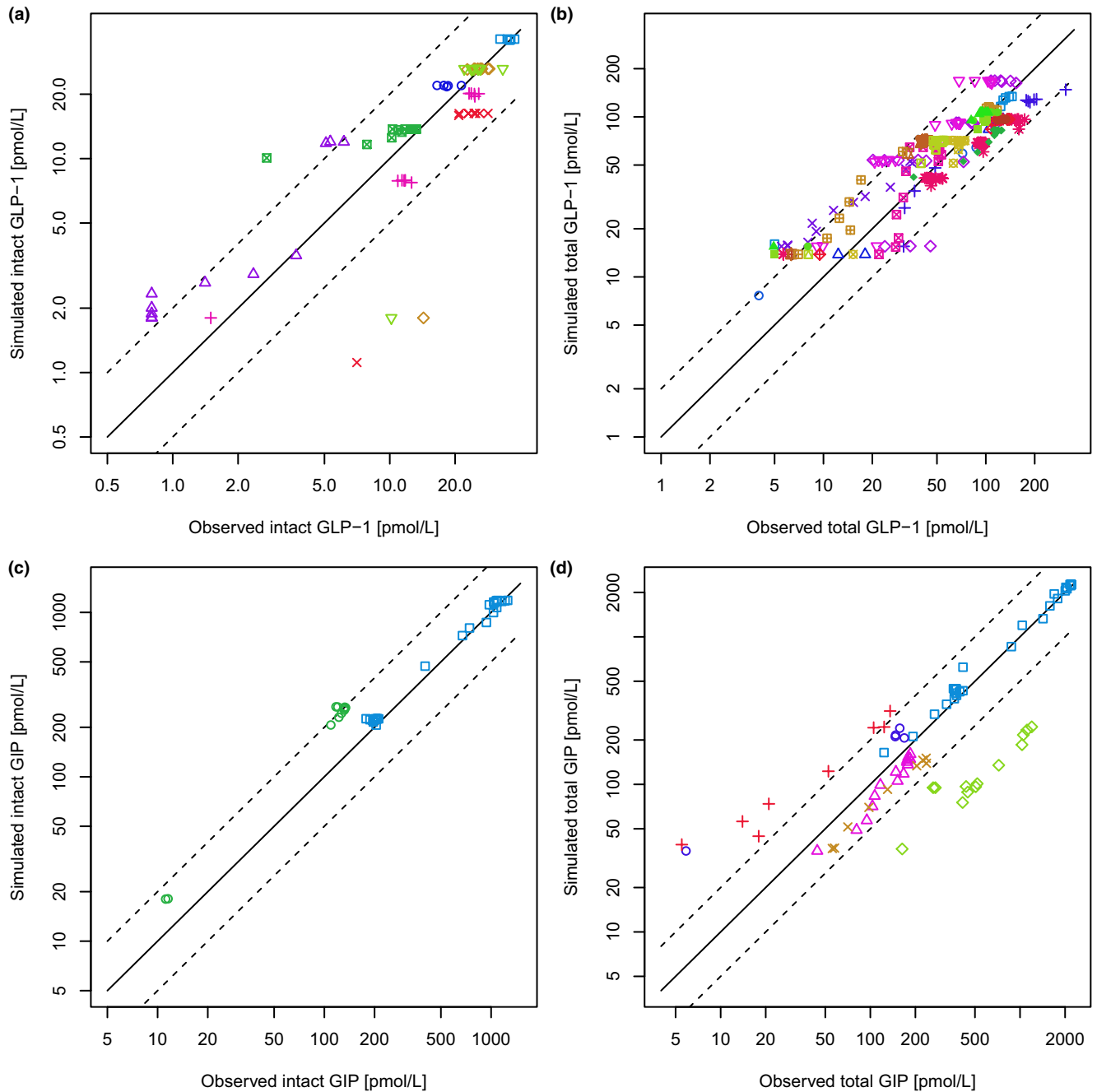
#### Incretin secretion

The previous sections describe processes of incretin metabolism based on data gathered from experiments with administration of exogenous GLP-1 or GIP. The following section focuses on endogenous production and secretion of the hormones.

GLP-1 and GIP are detected in human plasma in fasted conditions, implying a basal secretion of the hormones. The incretins are released into the interstitial space, with the secretion rate being calculated per L-cell or K-cell and multiplied by the number of respective cells in the mucosal segment. The numbers of endocrine cells per surface area mucosal tissue were extracted from literature,<sup>33</sup> and the values applied in the model are listed in **Table S9**.

GLP-1 and GIP are stored in a reserve pool (RP) after their synthesis, with the consequent transfer to a ready-to-release pool (RRP) and subsequent release into the interstitial space.

The secretion of GLP-1 is biphasic, with the first phase correlating with duodenal glucose delivery rates,<sup>34</sup> and the second phase depending on sodium/glucose cotransporter (SGLT)1-mediated rate of glucose absorption.<sup>35</sup> In the model, the rate of GLP-1 synthesis depends on the SGLT1-mediated glucose absorption, whereas the secretion rate (i.e., release of GLP-1 from the RRP) is increased by duodenal glucose presence. The transfer of GLP-1 between RP and RRP is not regulated.

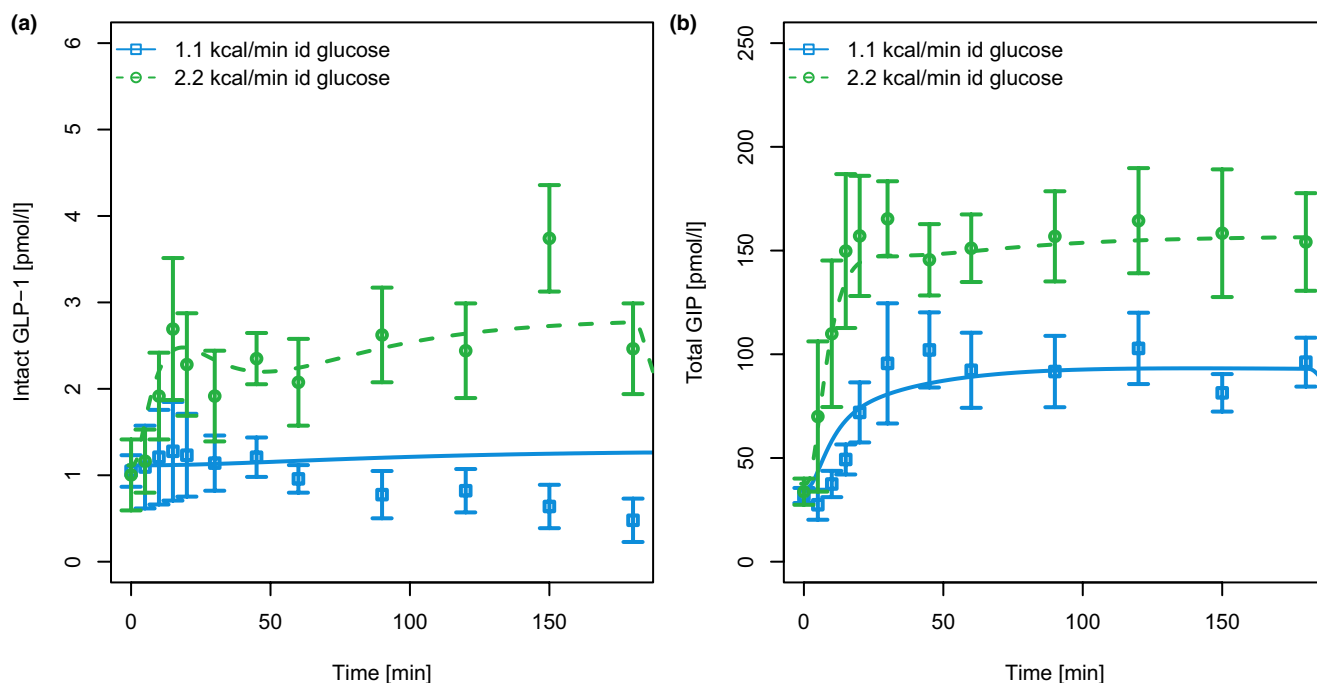


**Figure 3** Predictive performance of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) physiologically-based pharmacokinetic models. Comparison of simulated (y-axes) and observed (x-axes) concentrations of GLP-1 (7-36)amide (a), total GLP-1 (b), GIP (1-42) (c), and total GIP (d). A total of 35 data sets (Tables S3 and 43) were compared. The identity line represents the perfect accordance between simulated and observed data. The dashed lines above and below the identity line mark the 2-fold and 0.5-fold difference ranges, respectively.

Secretion of GIP is also stimulated by SGLT1-dependent glucose uptake.<sup>35</sup> To describe the observed data, glucose uptake additionally enhances GIP synthesis and the transfer between RP and RRP.

The model was fitted to intact GLP-1 and total GIP concentration-time profiles gathered during i.d. infusions of glucose at 1.1 and 2.2 kcal/min rates reported by Schirra *et al.*<sup>34</sup> The comparison of the simulation results and measured data

is presented in **Figure 4**. Data show a small initial increase in GLP-1 concentrations with consequent decrease below the basal levels during glucose infusion at a rate of 1.1 kcal/min. Although the initial increase is captured by the model (**Figure 4a**), the decrease below the basal levels cannot be simulated with the current structure. At a glucose delivery rate of 2.2 kcal/min, a peak rise in GLP-1 concentrations is observed during the first 15 minutes, with a consequent



**Figure 4** Secretion of the incretin hormones during intraduodenal glucose infusion. Comparison of simulated (lines) and observed (symbols) concentrations of incretins during 1.1 and 2.2 kcal/min intraduodenal infusions of glucose.<sup>33</sup> (a) Glucagon-like peptide-1 (GLP-1) (7-36)amide. (b) Total glucose-dependent insulinotropic polypeptide (GIP).

decrease of the concentrations and their stabilization at ~ 2.5 pmol/L. The model describes both, the initial peak and the observed plateau. In case of GIP (**Figure 4b**), the rise of hormone concentration is proportional to the rate of glucose infusion.

Reported experiments of i.d. glucose infusions (**Table S3**) were simulated to assess the predictive performance of the model. Although the general qualitative behavior of incretins secretion is well-captured, the absolute values differ between the simulations and reported data to various extents.

Notably, plasma concentrations of GLP-1 (**Figure S25**) declined below the basal levels at low glucose infusion rates.<sup>36–38</sup> Whereas the 2.2 kcal/min glucose infusion triggers a distinct GLP-1 response in the data set used for model development,<sup>34</sup> the 2 kcal/min infusion in the data set reported by Ma *et al.*<sup>37</sup> does not induce any steady-state increase in GLP-1 concentrations, and the values are below those of saline infusion. The model simulates a distinct response in the first 20 minutes, in accordance with the observations of Schirra *et al.*<sup>34</sup>

Predictions of GIP response to i.d. glucose (**Figure S26**) are quantitatively less reliable than the predictions of GLP-1 concentrations. Although the infusions reported by O'Donovan *et al.*<sup>38</sup> are predicted within a twofold difference, the data by Schirra *et al.*<sup>36</sup> and Ma *et al.*<sup>37</sup> are strongly overpredicted. However, Schirra *et al.*<sup>36</sup> do not clearly state whether the GIP assay measured the total or the active concentrations, and the simulated active concentrations are in accordance with the reported data, whereas the total concentrations were overpredicted by almost threefold (170 vs. 64 pmol/L). Ma *et al.*<sup>37</sup> reports unusually

low total GIP concentrations (8 pmol/L), and the basal simulated values are several-fold higher (22.8 pmol/L).

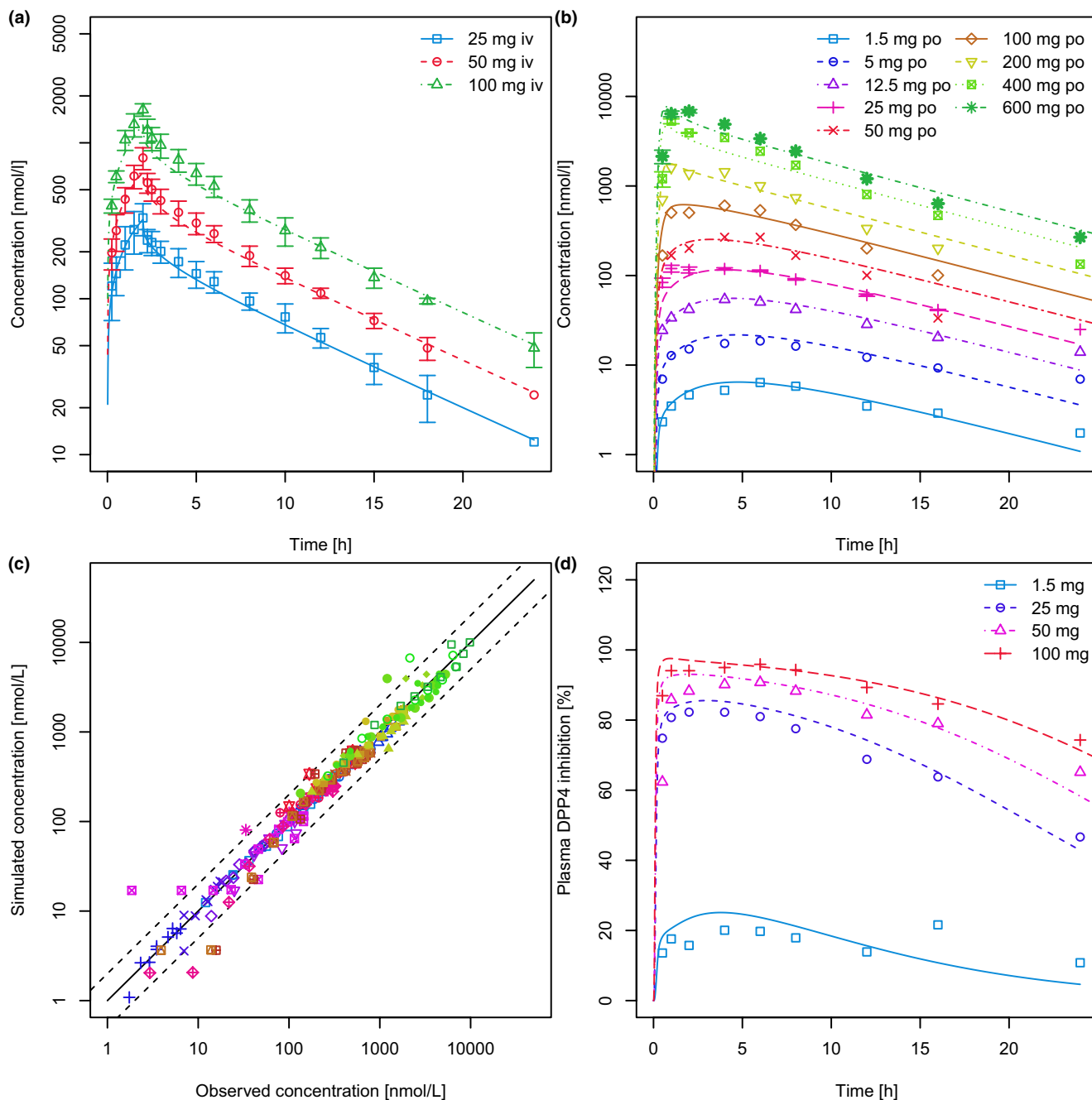
#### Model of sitagliptin PKs/PDs

The PBPK model of sitagliptin as a representative compound for the class of DPP4i was developed using information reported in literature and in the DrugBank<sup>33</sup> database (accession number DB01261). The model includes active transport of sitagliptin by the transporters P-gp and human organic cation transporter 3,<sup>39</sup> glomerular filtration, reversible binding to DPP4, as well as metabolism through cytochrome P450 3A4 (CYP3A4).

Although sitagliptin is reported to be highly soluble across the physiological pH range and the tablet formulation used in the final product is rapidly dissolving,<sup>40</sup> the reported concentration-time profiles show delayed absorption with plasma concentrations plateauing between 2 and 4 hours postdose at doses up to 200 mg.<sup>41</sup> Furthermore, ~ 11% of an 83 mg oral dose were excreted via feces.<sup>42</sup> A model with limited intestinal permeability in combination with P-gp-mediated efflux could not describe these observations.

We hypothesize that sitagliptin is reversibly bound to DPP4-like proteins in gut microbiota<sup>43</sup> prior to absorption, and traverses the lumen together with the microbiome. The model includes synthesis of DPP4-like proteins in lumen segments, reversible binding of sitagliptin, and transfer along the lumen.

Parameter values were identified by fitting the model to concentration-time profiles after i.v. and p.o. administrations of sitagliptin, the data sets are listed in **Table S4**. The model allows adequate description of sitagliptin PK (**Figure 5a,b**) with simulated 11% of 83 mg oral dose excreted to feces (observed 11%<sup>42</sup>) and 16% metabolized by



**Figure 5** Performance of the sitagliptin physiologically-based pharmacokinetic/pharmacodynamic model. (a) Comparison of simulated (lines) and observed (symbols) concentrations of sitagliptin after iv administration. Data from Bergman *et al.*<sup>49</sup> (b) Oral administration of sitagliptin. Data from Herman *et al.*<sup>41</sup> (c) Comparison of simulated (x-axis) and observed (y-axis) concentrations of sitagliptin. A total of 256 points from 23 data sets were compared. The identity line represents the perfect accordance between simulated and observed data. The dashed lines above and below the identity line mark the 2-fold and 0.5-fold difference ranges, respectively. (d) Comparison of observed and simulated inhibition (in % of basal activity) of plasma dipeptidyl-peptidase 4 (DPP4) after oral administration of sitagliptin.

CYP3A4 (reported total oxidative metabolism 16%<sup>42</sup>). The total comparison of 256 data points from 23 data sets is presented in **Figure 5c**, with 11 points (4%) being outside of the twofold difference range.

Inhibition of DPP4 by sitagliptin is modeled as reversible binding of the drug to the enzyme. The percentage inhibition of plasma DPP4 is modeled as the percentage of plasma

DPP4 bound to sitagliptin and is described well in dose-dependent manner (**Figure 5d**).

To assess the predictive performance of the pharmacological effect of sitagliptin, we simulated the study reported by Andersen *et al.*<sup>12</sup> Eight patients with T2DM received a continuous i.v. infusion of 1 pmol/kg BW/min GLP-1 followed by oral administration of 0 (placebo), 25, 100, or

200 mg sitagliptin. Arterialized venous plasma concentrations of active GLP-1 were measured.

The reported basal value of about 18 pmol/L active GLP-1 is unusually high, being a clear outlier compared with basal values reported in other sources (mean value of 3.9 pmol/L). The basal value simulated by the model is 1.8 pmol/L, and the reported value can only be simulated by disabling both the DPP4-mediated and NEP-mediated metabolization processes or increasing the basal secretion rate by more than 10-fold. We, therefore, decided to compare percentage increase in GLP-1 concentrations after administration of sitagliptin rather than the absolute values.

Administration of 25 mg sitagliptin increases the concentrations of GLP-1 by up to 51% (62 vs. 41 pmol/l) in the observed data, and by 63% in the simulations (36 vs. 22 pmol/l). The increase of the sitagliptin dose to 200 mg leads to only a minor further increase in peak GLP-1 concentrations (to 65 pmol/L). Consistent with these observations, the model predicts 4 pmol/l higher GLP-1 concentrations with administration of 200 mg compared with the administration of 25 mg sitagliptin, as shown in **Figure 6a**. **Figure 6b** presents the simulated effect of sitagliptin administration on the concentrations of active GIP during the infusions of 4 pmol/kg BW/min GIP. The increase of intact GIP concentrations during hormone infusion amounts to 54% and is comparable to that seen for GLP-1.

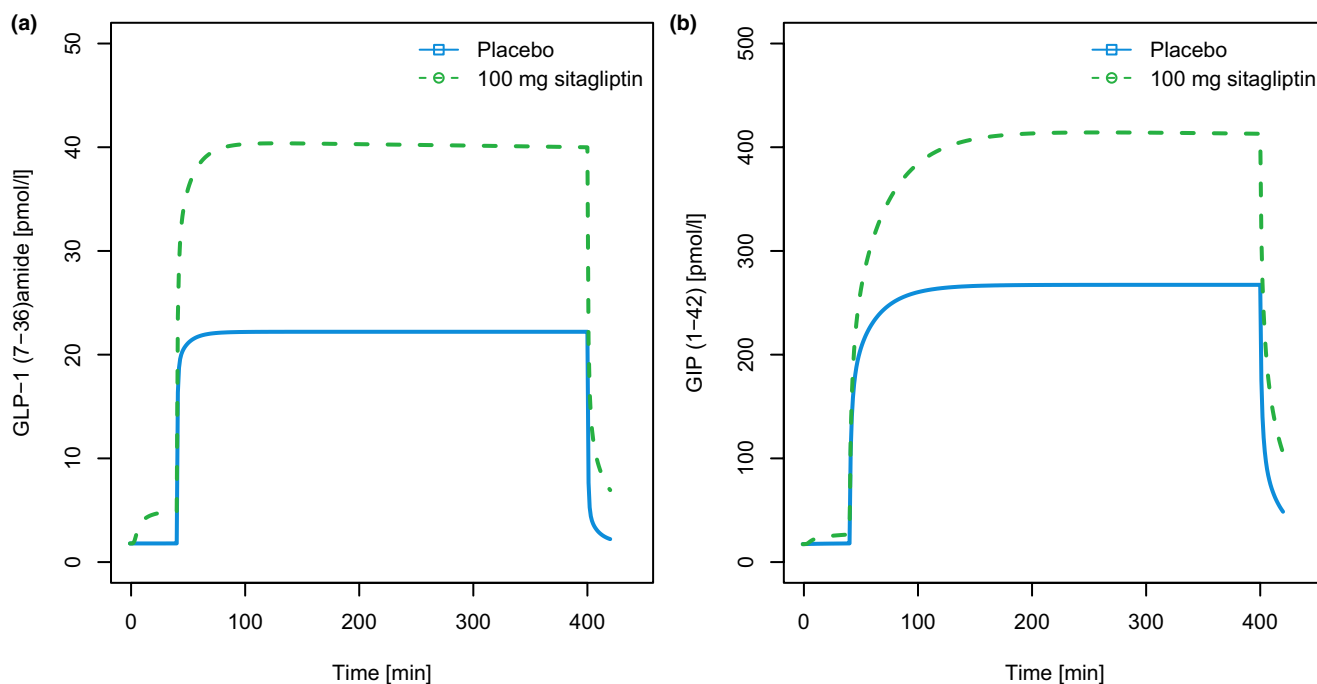
## DISCUSSION

Until recently, only the total or the active forms of GLP-1 could be measured, and it has been assumed that the total

form consists of GLP-1 (7-36)amide and the metabolite formed by DPP4. A recent advance in developing a GLP-1 (9-36)amide assay by Albrechtsen *et al.*<sup>13</sup> reveals the presence of a yet uncharacterized form. This discovery paves the way for better understanding of GLP-1 metabolism and development of more precise models. The observed concentrations of all three measured forms (GLP-1 (7-36)amide, GLP-1 (9-36)amide, and total GLP-1) can be described by a model incorporating the degradation of GLP-1 (7-36)amide and (9-36)amide by NEP, pointing to the potential importance of the enzyme.

During model development we were confronted with high heterogeneity of reported GLP-1 concentrations, (basal values between  $< 1^{34}$  and  $20^{12}$  pmol/L). This variability can be partly attested to the differences in the specificity and sensitivity of available assays,<sup>44</sup> whereas no biological factors could be identified. Because the modeled elimination processes scale linearly in the physiological concentration range, an underprediction or overprediction of an i.v.-infusion experiment reflects the differences between the data sets after dose normalization. Based on this assumption, we excluded the six data sets that reported steady-state concentrations that are  $> 100\%$  higher than dose-normalized (and thus predicted) from the analysis as "outliers." As the mean demographic characteristics between the groups did not markedly differ, reliable conclusions about the sources of the observed differences cannot be made and incorporated in the model.

Neither the cross-compatibility of the results nor the choice of a certain assay are discussed within the studies, and direct comparison of the observed results seems difficult. To assess the interindividual variability in GLP-1 levels



**Figure 6** Simulation of sitagliptin effect on incretin concentrations. Administration of 200 mg sitagliptin was simulated at time 0. At time 20 minutes, an infusion of 1 pmol/kg body weight (BW)/min glucagon-like peptide-1 (GLP-1) (a) or 4 pmol/kg BW/min glucose-dependent insulintropic polypeptide (GIP) (b) was initiated and lasted for 360 minutes. Shown is the simulated concentration in arterialized venous blood.



and to identify the causes, a consistent data set produced under similar conditions, including measurements of at least the active and the total forms, is required. We further conclude that studies assessing the metabolic state of an individual (e.g., an oral glucose tolerance test) should report both, the total and the intact concentrations of GLP-1. Because total GLP-1 concentrations are not affected by the activity of DPP4 (and, potentially, NEP), they can be used to derive the secretory capacity of an individual, whereas intact GLP-1 concentrations reflect the biologically active moiety. Only measuring the total GLP-1 concentrations, as it has been proposed,<sup>44</sup> would not reflect the variability observed in DPP4 activity.<sup>45</sup>

The challenges of model validation and variability assessment are especially true for the GIP PK model. Available data on GIP secretion is highly inconsistent.<sup>46</sup> With the limited data available, an accurate characterization of GIP metabolism is not possible. Only one data set<sup>27</sup> reports the concentrations of both, the active and total forms of GIP, but the metabolite has not been directly measured so far.

The presented model describes incretin responses to ingested glucose as the most prominent secretagogue. The responses to i.d. infusions of glucose reported by Schirra *et al.*<sup>34</sup> are accurately reproduced. This peak originates from the initially enhanced release of GLP-1 molecules from RRP vesicles, and the intensity of the peak is primarily determined by the size of the RRP. After the RRP is depleted, concentrations of GLP-1 slightly decrease, followed by a steady increase due to an increased synthesis rate. The secretion of GIP is proportional to the rate of glucose delivery and does not show a biphasic pattern.

The predictions of GLP-1 and GIP responses are in acceptable quantitative and qualitative agreement with data,<sup>36,38</sup> except for the several-fold overprediction of the GIP concentrations reported by Ma *et al.*<sup>37</sup> Although the cause of this overprediction cannot be identified, it can be described by varying the K-cell and L-cell density in the intestinal mucosa or the basal secretion rates. No study, however, reports the concentrations of both, the active and the total, forms of the incretins during i.d. glucose infusion, making it impossible to differentiate between the variability in incretin secretion and metabolism. Furthermore, variability in the positioning of the catheter for glucose infusion in the duodenum could introduce significant variability in the observations.

The mechanistic description of the degradation of incretins enables predictions of the effects of specific DPP4 inhibitors. We have modeled the PK of sitagliptin and incorporated its inhibitory effects on the plasma soluble and membrane bound DPP4. The model captures the dose-dependency of plasma DPP4 inhibition well, with 100 mg once daily being sufficient for a constant > 80% inhibition. The direct comparison of the predicted and observed pharmacological effect is not trivial as the only data set assessing the effect of sitagliptin on GLP-1 levels during GLP-1 infusion<sup>12</sup> reports unusually high basal and steady-state GLP-1 concentrations. The reasons for such high deviation from other reported concentrations is not clear, and the comparison of absolute values with simulations seems not to be reasonable. Nevertheless, the simulated percentage

increase in GLP-1 concentrations during i.v. infusion with sitagliptin administration at different doses is very close to the observed increase, which allows us to conclude that the model provides a suitable platform for predictions of DPP4i effects considering the high observed variability between the studies.

In summary, we present a model of incretin dynamics that incorporates the current knowledge of the underlying processes. The model offers a plausible and consistent description of incretin metabolism and secretion, and is the first of its kind that predicts concentrations of the various forms of incretins, including the undiscovered metabolite(s). We were not able to find any plausible physiological explanation for the variability in reported concentrations between studies and conclude that the major determinants are the choice of the assay and the uncertainties of the experimental procedures. Given a consistent (i.e., produced under similar conditions) set of data on patient level, including patient characteristic to inform the physiology, the model could be further extended to better leverage the PBPK framework and identify the interindividual variability of the modeled processes, offering a tool for population simulations. Inclusion of the metabolites in the model allows the distinction between the interindividual variability in incretin secretion and degradation and the relative contribution of the different metabolization pathways providing an explanation for the limited maximal effect of DPP4i. Furthermore, the metabolites allow to compare concentration data from different sources that only report either the active or the total form.

As the majority of data gathered in i.v. infusion experiments are predicted within the twofold range, we conclude that the model adequately represents the mean population. Despite the large quantitative uncertainty observed in i.d. glucose infusion experiments, the qualitative behavior of GLP-1 and GIP secretion and the effects of DPP4i are captured well, and the model can be considered reliable for predicting PD effects of the incretins and DPP4i. The model is freely available at <http://www.open-systems-pharmacology.org> and, in combination with the PBPK/PD model of the DPP4i sitagliptin and the previously developed model of SGLT2 inhibitors,<sup>47</sup> is well-suited for the integration into large scale diabetes models,<sup>47,48</sup> such as to allow more detailed simulations of glucose homeostasis and predictions of efficacy of treatment combinations in the context of translational and personalized medicine.

**Supporting Information.** Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website ([www.psp-journal.com](http://www.psp-journal.com)).

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**Conflict of Interest.** P.B. is currently employed by esqLABS GmbH and was an employee of Bayer AG during the time parts of the presented research were conducted. T.E. is an employee and stockholder of Bayer AG. All other authors declared no competing interests for this work.

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- Holst, J.J. On the physiology of GIP and GLP-1. *Horm. Metab. Res.* **36**, 747–754 (2004).
- Mentlein, R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul. Pept.* **85**, 9–24 (1999).
- Nargis, T. & Chakrabarti, P. Significance of circulatory DPP4 activity in metabolic diseases: circulatory DPP4 and metabolic diseases. *IUBMB Life* **70**, 112–119 (2018).
- Holst, J.J. The physiology of glucagon-like peptide 1. *Physiol. Rev.* **87**, 1409–1439 (2007).
- Hupe-Sodmann, K. et al. Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7–36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul. Pept.* **58**, 149–156 (1995).
- Plamboeck, A., Holst, J.J., Carr, R.D. & Deacon, C.F. Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. *Diabetologia* **48**, 1882–1890 (2005).
- Windeløv, J.A. et al. Why is it so difficult to measure glucagon-like peptide-1 in a mouse? *Diabetologia* **60**, 2066–2075 (2017).
- Hinnen, D. Dipeptidyl peptidase-4 inhibitors in diverse patient populations with type 2 diabetes. *Diabetes Educ.* **41**, 19S–31S (2015).
- Siegel, E.G. et al. Biological activity of GLP-1-analogues with N-terminal modifications. *Regul. Pept.* **79**, 93–102 (1999).
- Frias, J.P. et al. Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. *Lancet* **392**, 2180–2193 (2018).
- Idorn, T. et al. Elimination and degradation of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with end-stage renal disease. *J. Clin. Endocrinol. Metab.* **99**, 2457–2466 (2014).
- Andersen, E.S. et al. Is glucagon-like peptide-1 fully protected by the dipeptidyl peptidase 4 inhibitor sitagliptin when administered to patients with type 2 diabetes? *Diabetes Obes. Metab.* **20**, 1937–1943 (2018).
- Wewer Albrechtsen, N.J. et al. A sandwich ELISA for measurement of the primary glucagon-like peptide-1 metabolite. *Am. J. Physiol. Endocrinol. Metab.* **313**, E284–E291 (2017).
- Kuepfer, L. et al. Applied concepts in PBPK modeling: how to build a PBPK/PD model. *CPT Pharmacometrics Syst. Pharmacol.* **5**, 516–531 (2016).
- Open Systems Pharmacology Suite. <www.open-systems-pharmacology.org>.
- R Development Core Team. R: a language and environment for statistical computing. The R Project for Statistical Computing. <https://www.r-project.org/> (2011).
- Walløe, L. Arterio-venous anastomoses in the human skin and their role in temperature control. *Temperature* **3**, 92–103 (2016).
- Anoop, S. et al. High circulating plasma dipeptidyl peptidase-4 levels in non-obese Asian Indians with type 2 diabetes correlate with fasting insulin and LDL-C levels, triceps skinfolds, total intra-abdominal adipose tissue volume and presence of diabetes: a case-control study. *BMJ Open Diabetes Res. Care* **5**, e000393 (2017).
- Mazyar, J., Stanley, Z. & Wen-Tien, C. Plasma seprase and DPP4 levels as markers of disease and prognosis in cancer. *Dis. Markers* **32**, 309–320 (2012).
- Stenlid, R. et al. High DPP-4 concentrations in adolescents are associated with low intact GLP-1. *J. Clin. Endocrinol. Metab.* **103**, 2958–2966 (2018).
- Ahmed, R.H., Huri, H.Z., Al-Hamodi, Z., Salem, S.D. & Muniandy, S. Serum levels of soluble CD26/Dipeptidyl peptidase-IV in type 2 diabetes mellitus and its association with metabolic syndrome and therapy with antidiabetic agents in Malaysian subjects. *PLoS One* **10**, e0140618 (2015).
- Duke-Cohan, J.S., Morimoto, C., Rocker, J.A. & Schlossman, S.F. Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. *J. Immunol.* **156**, 1714–1721 (1996).
- Vella, A. et al. Effect of glucagon-like peptide 1(7–36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. *Diabetes* **49**, 611–617 (2000).
- Beglinger, C. et al. Pharmacokinetics and pharmacodynamic effects of oral GLP-1 and PYY3-36: a proof-of-concept study in healthy subjects. *Clin. Pharmacol. Ther.* **84**, 468–474 (2008).
- Koffert, J. et al. Effects of meal and incretins in the regulation of splanchnic blood flow. *Endocr. Connect.* **6**, 179–187 (2017).
- Toft-Nielsen, M.B., Madsbad, S. & Holst, J.J. Determinants of the effectiveness of glucagon-like peptide-1 in type 2 diabetes. *J. Clin. Endocrinol. Metab.* **86**, 3853–3860 (2001).
- Vilsbøll, T., Krarup, T., Madsbad, S. & Holst, J.J. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. *Diabetologia* **45**, 1111–1119 (2002).
- Christensen, M. et al. Glucose-dependent insulinotropic polypeptide augments glucagon responses to hypoglycemia in type 1 diabetes. *Diabetes* **64**, 72–78 (2015).
- Kreymann, B., Williams, G., Ghatei, M.A. & Bloom, S.R. Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* **2**, 1300–1304 (1987).
- Nauck, M. et al. Insulinotropic properties of synthetic human gastric inhibitory polypeptide in man: interactions with glucose, phenylalanine, and cholecystokinin-8. *J. Clin. Endocrinol. Metab.* **69**, 654–662 (1989).
- Lund, A., Vilsbøll, T., Bagger, J.I., Holst, J.J. & Knop, F.K. The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes. *Am. J. Physiol.-Endocrinol. Metab.* **300**, E1038–E1046 (2011).
- Nauck, M.A., Heimesaat, M.M., Holst, J.J., Ebert, R. & Creutzfeldt, W. Preserved incretin activity of glucagon-like peptide 1 [7–36 Amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes. *Mellitus* **91**, 301–307 (1993).
- Law, V. et al. DrugBank 4.0: Shedding new light on drug metabolism. *Nucleic Acids Res.* **42**, D1091–D1097 (2014).
- Schirra, J. et al. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J. Clin. Invest.* **97**, 92–103 (1996).
- Pais, R., Gribble, F.M. & Reimann, F. Stimulation of incretin secreting cells. *Ther. Adv. Endocrinol. Metab.* **7**, 24–42 (2016).
- Schirra, J. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. *Gut* **55**, 243–251 (2006).
- Ma, J. et al. Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes: glucose load and incretin responses. *Diabet. Med.* **29**, 604–608 (2012).
- O'Donovan, D.G. et al. Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. *J. Clin. Endocrinol. Metab.* **89**, 3431–3435 (2004).
- Chu, X.-Y. et al. Transport of the dipeptidyl peptidase-4 inhibitor sitagliptin by human organic anion transporter 3, organic anion transporting polypeptide 4C1, and multidrug resistance P-glycoprotein. *J. Pharmacol. Exp. Ther.* **321**, 673–683 (2007).
- US Food and Drug Administration. Sitagliptin Clinical Pharmacology and Biopharmaceutics Review(s). (U.S. Food and Drug Administration, Center for Drug Evaluation and Research, 2005).
- Herman, G. et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin. Pharmacol. Ther.* **78**, 675–688 (2005).
- Vincent, S.H. et al. Metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [14C]Sitagliptin in humans. *Drug Metab. Dispos.* **35**, 533–538 (2007).
- Olivares, M. et al. The potential role of the dipeptidyl peptidase-4-like activity from the gut microbiota on the host health. *Front. Microbiol.* **9**, 1900 (2018).
- Bak, M.J. et al. Specificity and sensitivity of commercially available assays for glucagon-like peptide-1 (GLP-1): implications for GLP-1 measurements in clinical studies. *Diabetes Obes. Metab.* **16**, 1155–1164 (2014).
- Ahmed, R.H. et al. Altered circulating concentrations of active glucagon-like peptide (GLP-1) and dipeptidyl peptidase 4 (DPP4) in obese subjects and their association with insulin resistance. *Clin. Biochem.* **50**, 746–749 (2017).
- Hoshiyama, A. et al. Identification of plasma binding proteins for glucose-dependent insulinotropic polypeptide. *Endocr. J.* **66**, 621–628 (2019).
- Balazki, P., Schaller, S., Eissing, T. & Lehr, T. A quantitative systems pharmacology kidney model of diabetes associated renal hyperfiltration and the effects of SGLT inhibitors. *CPT Pharmacometrics Syst. Pharmacol.* **7**, 788–797 (2018).
- Schaller, S. et al. A Generic integrated physiologically based whole-body model of the glucose-insulin-glucagon regulatory system. *CPT Pharmacometrics Syst. Pharmacol.* **2**, e65 (2013).
- Bergman, A. et al. Dose-proportionality of a final market image sitagliptin formulation, an oral dipeptidyl peptidase-4 inhibitor, in healthy volunteers. *Biopharm. Drug Dispos.* **28**, 307–313 (2007).

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