Citation: CPT Pharmacometrics Syst. Pharmacol. (2017) 6, 365–372; doi:10.1002/psp4.12189

ORIGINAL ARTICLE

A Population Dose–Response Model for Inhaled Technosphere Insulin Administered to Healthy Subjects

D Rüppel[1], R Dahmen[1], A Boss[2], R Jäger[1], M Grant[3], R Baughman[3] and T Klabunde[1]*

Technosphere insulin (TI), an inhaled insulin with a fast onset of action, provides a novel option for the control of prandial glucose. A euglycemic glucose clamp study was performed to compare the effects of TI and regular human insulin (RHI) on the induced glucose infusion rate (GIR) in healthy volunteers. Generation of a dose–response relationship between insulin dose and effect (expressed as AUC of GIR) was not possible from the clinical data directly. The GIR recording time was too short to capture the full effect and higher doses were not tested. Thus, a pharmacokinetic-GIR model was developed to simulate GIR for a sufficient time window of 20 h and for higher doses. A dose–response model was then generated from the simulated GIR profiles. The resulting model provides an ED_{50} for TI that is 5-fold higher than for RHI, a ratio that can be used as conversion factor for equivalent doses of RHI and TI.
CPT Pharmacometrics Syst. Pharmacol. (2017) **6**, 365–372; doi:10.1002/psp4.12189; published online 16 February 2017.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Technosphere insulin (TI), an inhaled insulin with a fast onset of action, provides a novel option for the control of prandial glucose.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The analysis quantifies the dose–response characteristics and pharmacodynamic behavior of TI with respect to onset and duration of action relative to subcutaneously administered regular human insulin (RHI).

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ The work illustrates how modeling and simulation can increase the value of incomplete experimental data. Unexpectedly, a linear dose–response behavior was found in the therapeutically relevant dose range.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ The simulations identify a 5:1 dose ratio of TI:RHI that yields equivalent pharmacodynamic effect. The ratio provides additional guidance to clinicians for switching between the two therapies. The faster onset and shorter duration of action also inform clinical use.

Intensive glycemic control often requires basal-bolus insulin treatment. However, hypoglycemia, weight gain, and the burden of multiple injections often lead to poor adherence.[1] An inhaled prandial insulin with rapid kinetics may address some of these concerns and could provide important therapeutic options for individualized diabetes management. Technosphere insulin (TI) (Afrezza MannKind, Valencia, CA) is a dry powder formulation of regular human insulin adsorbed onto Technosphere microparticles for oral inhalation.[2] Upon inhalation, these microparticles can reach the deep lung, allowing absorption into the systemic circulation with a time to maximum serum insulin concentration of 12–15 min.[3,4] TI is administered through the Afrezza inhaler device.[5]

Given that the pharmacokinetic (PK) profile of TI—with an early and high C_{max}—is different from the PK profile of subcutaneously (s.c.) administered regular human insulin (RHI), we wanted to quantify the dose–response relationship of TI and to compare it to the dose–response behavior of RHI:

- Is there a linear or curve-linear dose–response?
- At which dose does the linear relationship turn into curve-linear?
- Are the doses different among TI and RHI? This is important to support dosing recommendations when switching patients from regular human insulin to TI insulin.
- What would be the best starting dose?
- Is this conversion factor dependent on the initial RHI dose?

The PK and pharmacodynamics (PD) of TI was studied in 32 healthy volunteers (NCT01490762) in a hyperinsulinemic euglycemic glucose clamp crossover study.[6]

Insulin and glucose infusion rates (GIR) were measured to describe the PK profile and PD response. A limitation for a direct analysis from the data is that insulin measurements were taken only up to 3 h after dosing TI and GIR was measured only to 4 h; the full PD response was not covered especially for the higher TI doses (**Figure 1**). The limitations of the experiment may prevent accurate estimation of the curvature and maximum in a potential E_{max} dose–response model. Indeed, calculating the GIR_{AUC} as a marker for the PD response for different TI doses directly from the clinical data suggests a nonlinear dose–response relationship for the therapeutically relevant doses (up to 100 TI U).

[1]Sanofi-Aventis Deutschland GmbH, R&D Frankfurt, Germany; [2]Sanofi US, Bridgewater, New Jersey, USA; [3]MannKind Corporation, Danbury, Connecticut, USA. *Correspondence: T Klabunde (Thomas.Klabunde@sanofi.com)
Received 9 December 2016; accepted 16 February 2017; published online on 16 February 2017. doi:10.1002/psp4.12189

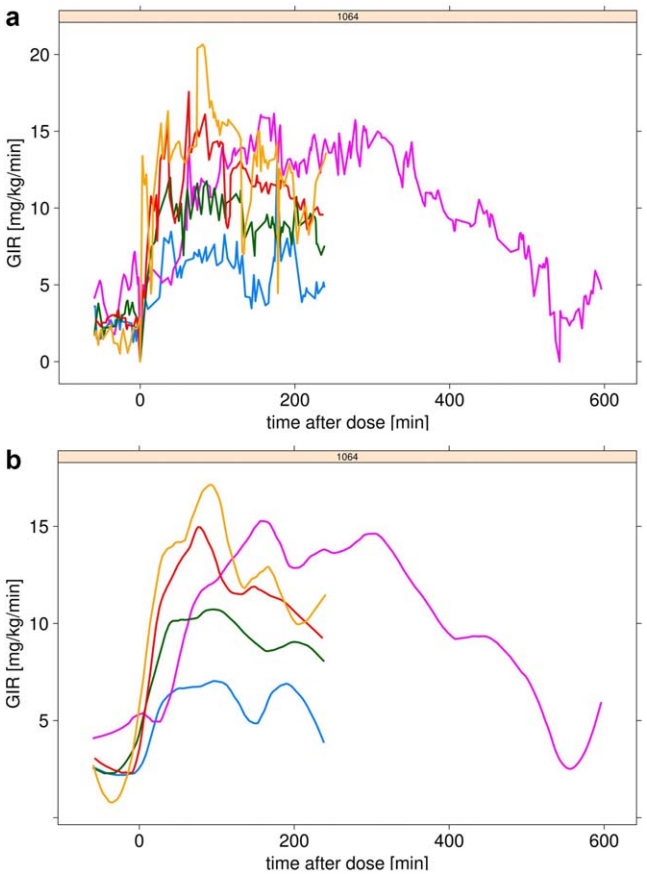

Figure 1 Typical time-course of glucose infusion rate (GIR) shown for one patient. GIR curves for four doses of TI insulin and for regular human insulin (RHI) are shown in different colors. The raw data (shown in **a**) with GIR being adjusted every minute reflects the noisiness of the experimental clinical data. The smoothed data (shown in **b**) reflects that for TI insulin the chosen recording time of 4 h is not sufficient to capture the full pharmacodynamic effect. Especially for the higher doses, a significant part of the PD, expressed as the area under the GIR curve, is lost, leading to an apparent saturation at the clinical doses tested.

Here we present the generation and application of a population PK/PD model. The analysis quantifies the dose–response characteristics and pharmacodynamic behavior of TI insulin with respect to onset and duration of action relative to subcutaneously administered RHI. The work also should illustrate how modeling and simulation can increase the value of incomplete experimental data. The main findings from this analysis have been presented as a poster at the ADA2016.[7]

METHODS

The objective of the analysis was to establish a simple mathematical description of the dose–response behavior (expressed as effect on GIR_{AUC}) for both insulins. This was not directly possible from the study data due to the "truncation" of the clamp data and due to the fact that higher doses were not tested. A much richer and more flexible PK-GIR model was therefore developed. **Figure 2** shows the approach to achieve the desired characterization of TI and RHI with respect to the dose–response and timing of the PD effect. The data from the study were used to develop a dynamic population PK/PD model relating insulin concentrations from an effect compartment to GIR (PK-GIR model). Although the dose–response model is already *implicit* in the construct of the (dose)-PK-GIR model, integrating the GIR_{AUC} analytically to relate dose to GIR_{AUC} response seemed to be very complicated, if not impossible. Thus, the model was used to simulate GIR curves beyond the experimentally recorded times up to 20 h to allow GIR values to return to baseline and the full PD effect of TI could be estimated. With this, the GIR_{AUC} response vs. dose can be plotted in order to receive a graphical dose–response relationship including higher doses and a 20-h integration time. However, although a figure captures the dose–response relationship graphically for the "dose, simulated GIR-AUC pairs," it does not provide a simple analytical mathematical relationship. Therefore, in a second step, we fit these pairs with several models, ending up with an E_{max}/ED_{50} model for both insulins. In other words, the second fitting was used to find an approximate analytical expression for the dose–response relationship in a situation

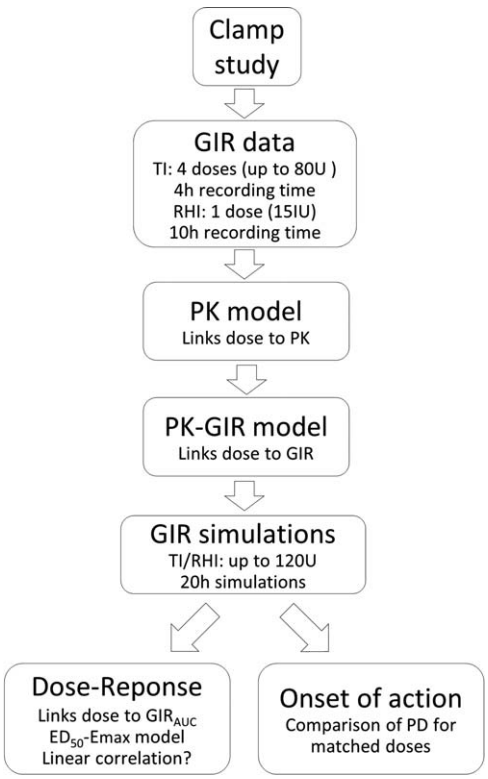

Figure 2 Model building procedure. The aim of the analysis is to use a PK/PD model based on data from an euglycemic glucose clamp to 1) quantify the dose relationship for TI in comparison to subcutaneously administered regular human insulin (RHI) and 2) compare the onset and duration of action of TI and RHI.

where analytical integration of the full (dose)-PK-GIR model seemed to be impossible.

The dose–response model allowed capturing the relative potency of both insulins by comparison of the ED_{50} values and to establish a simple relationship for dose pairs of TI and RHI with a matching response in terms of the AUC of GIR (e.g., 8IU dose of RHI and 40U dose of TI). The GIR time curves were simulated for RHI doses and TI doses providing an equivalent overall PD effect (as expressed by GIR_{AUC}) and could be compared with respect to the onset and duration of action and the time needed to reach half of the overall PD effect.

Study design and population

The protocol of study NCT01490762 was approved by the Independent Investigational Review Board on November 8, 2011 and the study-specific informed consent form was approved on November 14, 2011. All subjects provided their written informed consent before initiation of any study-related procedures. The study was conducted at the Profil Institute for Clinical Research (Chula Vista, CA). The study was sponsored by MannKind (Paramus, NJ). In study NCT01490762, 32 healthy volunteers received single doses of 10U, 30U, 60U, 80U TI, and 15IU RHI s.c. in a crossover design.[6] Insulin concentrations were measured predose and serially following administration of study treatments. C-peptide concentrations were measured as a marker for endogenous insulin secretion. For each treatment, total insulin concentration was recorded and the endogenous insulin concentration was estimated from the C-peptide concentration. The exogenous TI or RHI insulin was calculated by substracting the endogenous insulin from the measured insulin concentration. For each dose a hyperinsulinemic euglycemic clamp experiment was performed: After an overnight fast, insulin (insulin Lispro) is infused intravenously at a constant rate, resulting in a steady-state insulin level that is above the fasting levels. A glucose analyzer was used to frequently monitor blood glucose levels, while 20% dextrose was given intravenously at a variable rate to "clamp" blood glucose concentrations in the normal range. The glucose infusion rate was recorded before and several hours after dosage of RHI or TI. As hepatic glucose production is suppressed due to the hyperinsulinemic insulin levels and since there is no net change in blood glucose concentrations under steady-state clamp conditions, the GIR must be equal to the glucose disposal rate as a response to the exogenous insulin. After administration of the respective investigational drug, GIRs to maintain euglycemia were recorded over 4 h for TI and over 10 h for s.c. RHI. An example of the resulting GIR time course in one subject after receiving an insulin dose is shown in **Figure 1.** From the 31 subjects included in the modeling process, 18 were male, 13 female. Their weights ranged from 49–100 kg; their age was between 18 and 53 years.

Software

Mixed-effects modeling was performed using NONMEM (v. 7.2) with the FOCE INTERACTION approximation. Data management and diagnostics were performed using the statistical language R. PSN (Pearl Speaks NONMEM, Uppsala) was used to generate the visual predictive checks (VPC).

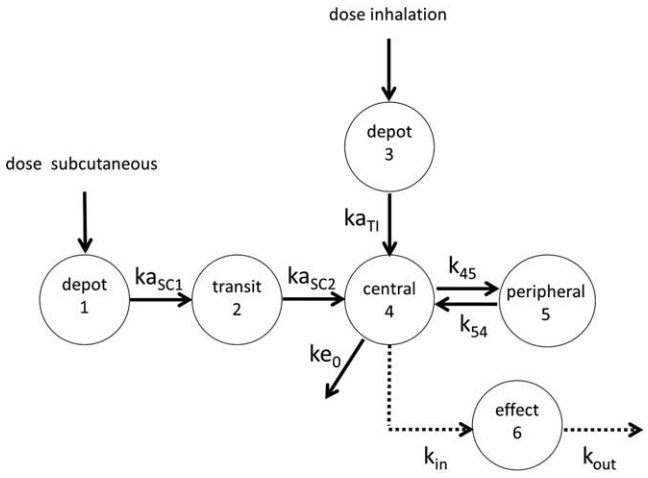

Figure 3 Pharmacokinetic model of insulin. ka_{SC1}, ka_{SC2} first-order absorption rates of RHI; ka_{TI} first-order absorption rate of TI; ke_0 elimination rate from central compartment; k_{45}, k_{54} exchange rates with peripheral compartment; k_{in}, k_{out} exchange rates for effect compartment.

Mixed-effects models

PK and PD models were developed as mixed-effects models (population models), i.e., deterministic parameters and random parameters (describing the distribution in the population) were fitted together in one step. Random parameters were modeled in exponential form with fixed effect p_0 and random effect η:

$$p = p_0 * e^{\eta_i} \quad (1)$$

Nested models were accepted as a better model if the objective function was 3.84 points lower ($P < 0.05$) when including a new parameter or covariate. In the covariate analysis a covariate was accepted if the objective function increased by 6.7 ($P < 0.01$) when the covariate was excluded from the model.

Population PK model

In order to capture the PK profiles of both insulins, a population PK model previously described by Potocka *et al.*[8] was used. We adapted the model for the data available from the clinical study. Insulin concentrations corrected by the C-peptide values were fit by NONMEM in the FOCE approximation to a structural model published previously (**Figure 3**). The model consists of a two-compartment model for distribution with a first-order elimination. Absorption of inhaled insulin was described by a first-order process. Absorption of RHI was modeled with two successive compartments with first-order absorption. A combination of additive and proportional error was used as an error model. Bioavailability of inhalable insulin F_{TI} is relative to subcutaneous application.

PK-GIR model

No smoother was applied and data were fitted directly with NONMEM. Glucose infusion rates as a function of insulin concentrations were modeled as an E_{max} model already published for this purpose.[9,10]

$$GIR = GIR_0 + \frac{GIR_{max} * c^{\gamma}}{EC_{50}{}^{\gamma} + c^{\gamma}}. \quad (2)$$

The model was developed in a two-step procedure. Individual PK parameters (*post-hoc* estimates) were used to predict concentrations at every timepoint necessary for the integration of the PK-GIR model. Random parameters were introduced in exponential form. Data were extremely rich in information, with about 1,800 observations per subject. FOCE INTERACTION was applied with the exception of etas characterizing the interoccasion variability of the baseline (HYBRID). An additive error was used as an error model. An effect compartment was necessary to describe the delay between effect and insulin concentrations.

Dose–response model (dose-GIR_{AUC} model)

The PK-GIR model described above was used to simulate GIR curves beyond the experimentally recorded times up to 20 h to allow the simulation of higher doses and the return of GIR values to baseline. Doses of up to 240U for TI and up to 80IU for RHI were simulated to fully capture the dose–response behavior by using the individual *post-hoc* parameter estimates from the PK-GIR model. From the resulting GIR(t) curves the AUC was calculated for the time window between 0 and 20 h for each dose/patient. Population dose–response models (dose-GIR_{AUC}) were developed relating insulin doses with the AUC of GIR. GIR-AUCs were modeled as a function of dose:

$$GIR_{AUC} = \frac{GIR_{AUC,max} * dose^{\gamma}}{ED_{50}{}^{\gamma} + dose^{\gamma}} \quad (3)$$

ED_{50} and $GIR_{AUC,max}$ are not independent in E_{max} models. Fitting is problematic without sufficient observations near E_{max} and can be significantly improved by using prior information. The maximal GIR for each patient $GIR_{max,i}$ is known from the PK-GIR model. With an integration time of 20 h the maximal area under the GIR time course can easily be calculated as the product of the maximal GIR for each patient (value at saturation) and the integration time:

$$GIR_{AUC,max,i} = GIR_{max,i} * 20h \quad (4)$$

The central value for $GIR_{AUC,max}$ was found to be 11.9 mg/kg/min *1200min = 14.3g/kg (g refers to glucose, kg to body weight).

$GIR_{AUC,max}$ was therefore not fitted but calculated individually and introduced as a covariate.

Random parameters were introduced in exponential form. A proportional error was used as error model. Fits were performed with the FOCE INTERACTION approximation.

RESULTS

Pharmacokinetics

In order to capture the PK profiles of both insulins a population PK model previously described by Potocka *et al.*[8] was used (**Figure 3**). All five dosing occasions were fitted together. One subject was excluded from the analysis, showing occasionally very high exposure impeding proper regression analysis. The VPC plot for all four doses of TI and one dose of RHI are shown as **Supplemental Figure S1**. The VPC indicates that the model correctly describes the central tendency. Variability in some cases tends to be either over or under observations. As the data did not include i.v. measurements, the elimination rate of insulin could not be fitted, but was taken from Ref. 8.

Table 1 Population parameters of PK-GIR model

Parameters	Estimate (95% CI)
Fixed effects	
GIR_0 (mg/kg/min)	2.7 (2.3–3.1)
GIR_{max} (mg/kg/min)	11.9 (–)
EC_{50} (mU/L)	152 (130–174)
γ (1)	1.8 (1.5–2.1)
K_{in}(1/h)	1.24 (1.08–1.39)
K_{out}(1/h)	1.24 (1.08–1.39)
Random effects	
ω_{Emax} (%)	31 (21–38)
ω_{EC50} (%)	38 (23–49)
IOV_{GIR0} (%)	61 (51–70)
σ_A (mg/kg/min)	2.1 (1.9–2.4)

Source: Fit119360.
PK, pharmacokinetic; GIR, glucose infusion rate; GIR_0, baseline; GIR_{max}, maximum GIR; EC_{50}, concentration of half maximum effect in effect compartment; γ, Hill coefficient; k_{in}, k_{out}, exchange rates for effect compartment; ω_{Emax}, ω_{EC50}, intersubject variability of fixed effects parameters; IOV_{GIR0}, inter occasion variability on GIR_0; σ_A, intrasubject variability, additional error; CI, confidence interval.

The resulting PK parameters of insulin are listed in **Supplementary Table S1** and are within the 95% confidence interval with the previously reported values in Ref. 8, with the exception of $k_{a,TI}$. This difference could be most likely attributed to different inhaler versions used in both studies.

Please also note that the bioavailability of TI insulin of 25% (21%,28%) identified in this study is relative to RHI, as no absolute bioavailabilty could be quantified in the absence of insulin i.v. data. With a bioavailabilty of RHI determined to 53%[8] the absolute bioavailability of TI found in this study can be estimated to 12–13%. This is in good agreement with the bioavailability of TI of 11% found in Ref. 8. Individual pharmacokinetic parameters were used in the PK-PD development.

PK-GIR model

The GIR curves show a significant noise with a high interoccasion variability for the baseline GIR. In **Figure 1** the GIR curves of one example subject are given, each showing the GIR curves from the five different occasions (four doses TI, one dose RHI). As data were not baseline-corrected, the baseline was part of the fit. The population GIR_{max} was determined by profiling, starting with a value from Ref. 10. The result of the profiling is shown in **Supplementary Material Figure S2**. Individual GIR_{max} and their distribution were fit. Results for the fit parameters are documented in **Table 1.** The additive error of the fit seems to be comparable to the baseline. The results from

Table 2 Population parameters of the dose-GIR_{AUC} model (mean and standard deviation are given)

Parameters	Estimate (95% CI) TI	Estimate (95% CI) RHI	Estimate (95%CI) TI/RHI*
Fixed effects			
$ED_{50,TI}$ (U)	249 (202–296)		245 (201–290)
$ED_{50,RHI}$ (IU)		43 (36–51)	48 (39–57)
γ (1)	1.07 (0.98–1.15)	1.15 (1.08–1.23)	1.08 (0.99–1.16)
Random effects			
$\omega_{ED50,TI}$ (%)	40 (24–52)		40 (25–51)
$\omega_{ED50,RHI}$ (%)		49 (40–56)	45 (35–54)
ω_{γ} (%)	17 (9–23)	17 (12–21)	17 (10–22)
σ_p (%)	22 (20–25)	4 (3–5)	21 (18–23)
Source	Fit 626954	Fit 629531	Fit 47694

ED_{50}, dose of half maximum effect; γ, Hill coefficient; ω_{ED50}, ω_{γ} intersubject variability of fixed effects; σ_p, intrasubject variability, proportional error; CI, confidence interval.
*Common fit of TI and RHI with same Hill coefficient.

a VPC stratified by dose are shown in **Supplemental Figure S3A–E**. The 15IU dose in **Figure S3B** corresponds to RHI, all other doses refer to TI. The VPC indicates that the model correctly describes the central tendency, but slightly overpredicts the variability for higher doses. The variability of the baseline in the beginning is overpredicted. The baseline is fitted over the whole time of the experiment. The contribution before the experiment is certainly higher than during the period under exogenous insulin. However, a positive or negative drift of the baseline during the experiment will influence the fitted baseline, which may become higher or lower than in the beginning. Our interpretation is that the variability in addition to what was observed in the beginning is influenced by drifting baselines.

Dose–response model (dose-GIR_{AUC} model)

The objective of the analysis was to establish a simple mathematical description of the relative dose–response behavior for both insulins. Due to the limitations of the experimental data, which were 1) limited to testing lower doses of TI insulin and 2) not allowing GIR curves to return to baseline, thus truncating parts of the effect, the PK-GIR model was used to simulate GIR curves beyond the experimentally recorded times up to 20 h to allow the simulation of higher doses and the return of GIR values to baseline. GIR curves were simulated for all 31 healthy subjects using the individual *post-hoc* estimates from the PK-GIR model for doses up to 240U for TI and up to 80IU for RHI and times up to 20 h. The AUC of the GIR was integrated up to 20 h and fit with the dose-GIR_{AUC} model described above (**Table 2**). $GIR_{AUC,max}$ is not a fit parameter but calculated individually according to Eq. 4. Results from a visual predictive check for RHI and TI are shown in **Supplemental Figure S4.** Mean GIR_{AUC} as a function of dose for TI and RHI is shown in **Figure 4a.** TI reached its half-maximal effect ED_{50} at a dose of 249U and RHI at a dose of 43IU. The higher ED_{50} of TI reflects its lower bioavailability and its higher, sharper peak resulting in a reduced PD action at a given dose.

From our work it is evident that the time window used for integration of the GIR time profiles is important and that short integration times (e.g., 4 h) not allowing to capture the full pharmacodynamic effect lead to an apparent saturation as part of the effect is truncated for high doses. This is reflected in **Figure 4b.** Here the dose-GIR_{AUC} relationship is shown for RHI and TI based on simulated GIR time curves using an integration time of 4 h. The ED_{50}s are much lower (RHI: 20IU, TI: 35U), indicating an artificial apparent saturation of the response at much lower doses.

Doses of TI and RHI producing the same GIR_{AUC} are considered equivalent. Due to the fact that the Hill coefficients for TI and RHI dose–response model are almost the same, a simple conversion rule for equivalent TI and RHI doses can be derived from Eq. 3:

$$dose_{TI} = ED_{50,TI} / ED_{50,RHI} * dose_{RHI} \quad (5)$$

In order to establish this relationship we also fit the dose–response of TI and RHI using a common Hill coefficient (**Table 2,** right column). Here we obtained ED_{50} values of

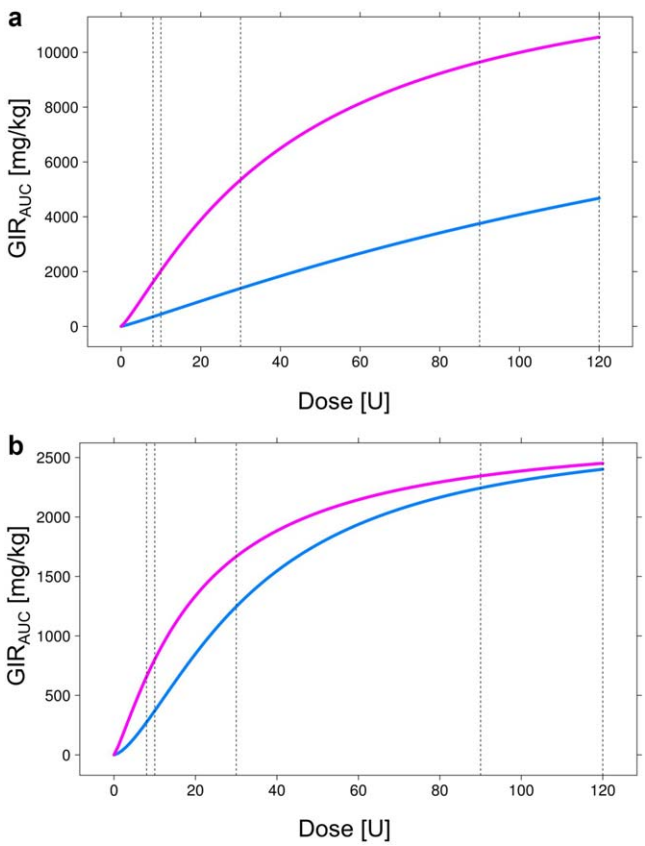

Figure 4 (**a**) GIR_{AUC} as a function of dose for integration time of 20 h (TI: blue, RHI: magenta). The maximum GIR_{AUC} is 13.8 g/kg; the dose of half maximum effect for TI ED_{50} is 249U; the ED_{50} for RHI is 43IU. Dotted lines corresponded to 8, 10, 30, 90, 120U insulin. Linear ranges are 0 to 120U for TI and 0 to 30IU for RHI. (**b**) For comparison the GIR_{AUC} as function of dose is shown for an integration time of 4 h. Apparent saturation due to a short integration window becomes visible at lower doses with ED_{50} for RHI of 20IU and for TI of 35U.

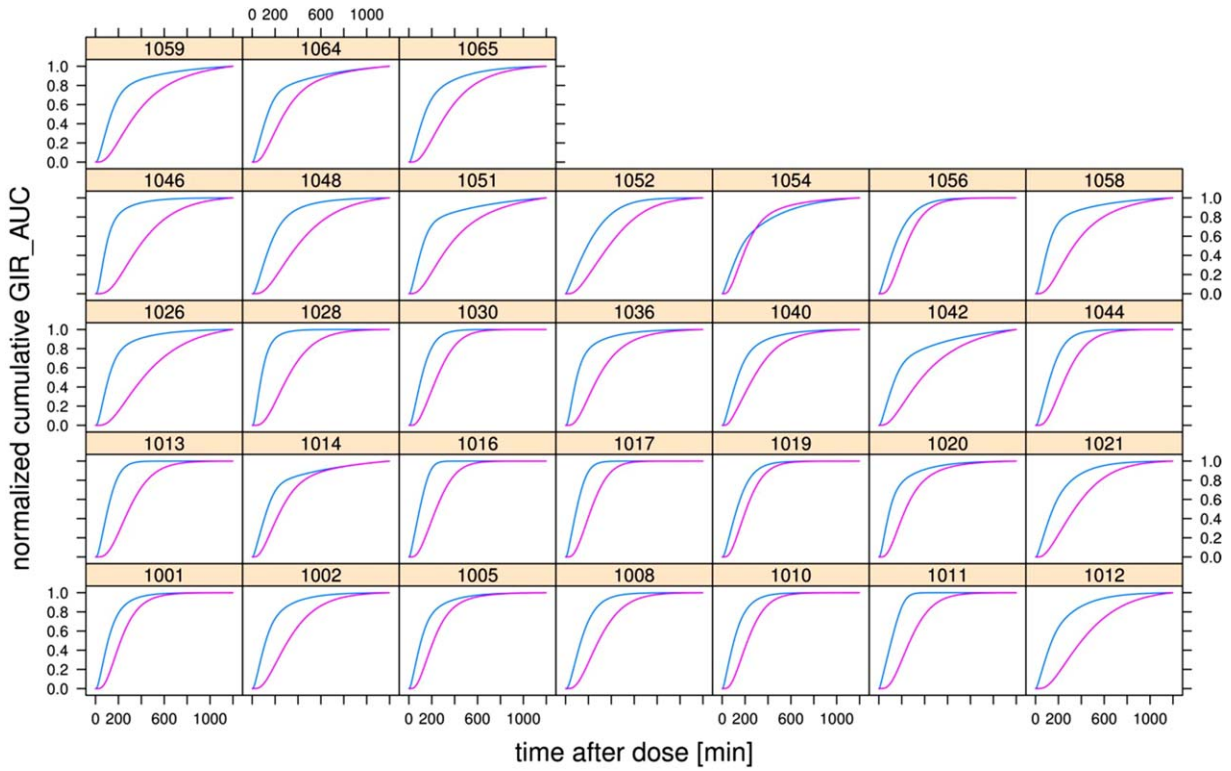

Figure 5 Cumulative GIR_{AUC} per individual from study NCT01490762 for 8IU RHI and 40U TI, considered as equivalent (TI: blue, RHI: magenta). The cumulative GIR_{AUC} is normalized to GIR integrated over 20 h.

245U for TI and 48IU for RHI. The ratio $ED_{50,TI}/ED_{50,RHI}$ was calculated to 5.1 and Eq. 5 with this factor applies for all doses.

Cumulative GIR_{AUC} and comparison of onset of action

In order to evaluate the onset of action for equivalent TI and RHI doses (same GIR_{AUC}), cumulative AUCs of simulated GIR profiles were calculated and normalized to GIR integrated over 20 h.

$$GIR_{AUC,cum}(t) = \frac{\int_0^t GIR(t)\,dt}{\int_0^{20h} GIR(t)\,dt} \quad (6)$$

Curves for the equivalent doses 8IU RHI and 40U TI are shown in **Figure 5.** TI has a steeper increase of $GIR_{AUC,cum}$ than RHI in each individual time course. Times to reach 10%, 50%, and 90% of the individual total PD effect, measures of both onset and duration, are shorter for TI than for RHI are given in **Supplemental Table 2.** The steeper cumulative GIR_{AUC} time curves reflect a faster onset of action ($T_{10\%}$-$GIR_{AUC,0\text{-}20\ h}$: RHI, 122 ± 23 min; TI, 35 ± 5 min) and a shorter duration of action ($T_{90\%}$-$GIR_{AUC,0\text{-}20\ h}$: RHI, 621 ± 152; TI, 386 ± 136 min). Time to reach half of the overall effect was shorter for TI ($T_{50\%}$-$GIR_{AUC,0\text{-}20\ h}$: RHI, 296 ± 64 min vs. TI, 124 ± 25 min).

DISCUSSION

TI, an inhaled human insulin with a fast onset of action, provides a novel option for postprandial glucose control. However, the sharp and high insulin peaks after TI dosing have raised concerns that its PD effects (e.g., glucose disposal) might be saturated at doses within the therapeutic range. This would contrast with regular human insulin or prandial insulin analogs administered via the s.c. route, where saturation is only seen beyond the therapeutic range. Differences in the dose–response behavior, in bioavailability, and in the time-action profiles between RHI and TI might lead to complex dose conversion rules for patients switching from TI to RHI or vice versa. Thus, it is of utmost importance to compare the dose–response relationships of TI and RHI.

Here the results from an euglycemic clamp study in healthy volunteers were used to compare the effects of TI and RHI on the induced GIR time curves at various doses. Since the study examined only a single dose of RHI and up to only 80U for TI, a population PK/PD model (PK-GIR model) was generated and applied to simulate GIR curves for an extended range of doses and to capture the full PD effects over 20 h (instead of 4 h for TI and up to 10 h for RHI, as in the clamp study apparently truncating parts of the PD effect; **Figure 1**). We are confident that our concentration-effect model (PK-GIR model) is sufficiently

robust for extrapolation to higher doses and times. It is relatively simple and based on thousands of observations covering a concentration range that generates glucose infusion rates from zero to GIR_{max}, thus also describing the saturation of the effect on the GIR. In addition, the model is not time-dependent and assumes quick equilibration of the insulin/glucose relationship (steady state, as assumed in GIR experiments, measurements up to 24 h reported). Concentrations and their time courses are described by the PK models for the TI and s.c. formulations. The disposition part is identical for both formulations; differences for the absorption process (s.c. vs. inhaled) are covered. As TI and s.c. formulations both show dose-proportional exposure of clinically relevant doses, the model appears to be robust enough to extrapolate in time and dose within the therapeutical dose range. The PK of TI was tested up to 80U in this study, but recently up to 120U in a recent clamp study performed in type-1 patients (NCT02470637). The results have been shown at ADA2016 and describe the C_{max} and AUC of TI as dose-proportional.[12] Subcutaneously applied human insulin was tested up to 30U and found to be dose-proportional.[13] Beyond these limits, deviation from dose-linearity may occur and the model should be considered a hypothesis. Extrapolation to higher doses was nevertheless necessary to fit the dose effect relationship, especially the ED_{50}. The relationship is therefore valid in the limits of the known PK dose linearity, which is approximately the therapeutically relevant dose range.

The objective of the analysis was to establish a simple mathematical description of the dose-GIR_{AUC} response behavior for both insulins. GIR curves were simulated for all 31 healthy subjects using the PK-GIR model for doses up to 240U for TI and up to 80IU for RHI and times up to 20 h. The resulting AUC of the GIR curves were used to fit a dose-GIR_{AUC} model. Not surprisingly, an ED_{50}/E_{max} model was best able to describe the observed dose–response for TI and RHI covering a wider dose range, indicating saturation of the overall PD effect at very high doses. Unexpectedly, the dose-GIR_{AUC} for TI appeared to be almost linear for lower doses of TI (10–120U), indicating that in the therapeutically relevant dose range the dose–response of TI and RHI is dose linear (**Figure 4a**).

The dose needed to achieve the half-maximal effect was 245U for TI compared to 48IU for RHI. The factors reducing the PD effect of TI are its lower bioavailability, higher C_{max}, and shorter absorption half-life. A simple dose conversion rule could be generated from the ratio of these ED_{50}, suggesting that a 5.1-fold higher dose of TI matches the PD effect of a given RHI dose. A similar effect factor is evident in a clinical phase III study in type-1 patients comparing the treatment effects on HbA1c for TI and insulin aspart.[11] After 24 weeks of treatment the average daily dose of insulin aspart 25.8U and the average daily dose of TI was 115.4U (factor 4.5).

In spite of limitations of the present population PK/PD and simulation approach—extrapolating in time and dose—to characterize the dose–response relationship of inhaled TI insulin and RHI, it needs to be mentioned that our findings are in line with the analysis of a very recent PK/PD clamp study of inhaled TI insulin in type-1 patients (NCT02470637).[12] In the recent study, inhaled TI doses of up to 120U and up to 90U insulin Lispro were tested in type-1 patients with a GIR recording time of 12 h. In this study a dose-proportional response was found for TI doses of up to 120U and a dose–response ratio of TI:Lispro of ~6.5 was found (ED_{50} of 51U for insulin lispro and 332.5 TIU for inhaled TI).

One might argue that for a prandial insulin like TI, a shorter PD window (e.g., 4 or 6 h) is physiologically more meaningful. To define the full dose-GIR_{AUC} curve, however, supratherapeutic doses were required and shortening the integration time truncated the GIR curves and introduced an artifact into the results: an apparent saturation of the PD effect within the therapeutic range. Using a 4 h integration time the ED_{50} become smaller and the response relationship turns from linear to curve-linear at therapeutically relevant doses (ED_{50} ratio of 1.75).

To compare the timing of the pharmacodynamic activity of RHI and TI, we compared simulated GIR-time curves for doses that produced the same GIR_{AUC}. Specifically, we compared an RHI dose of 8IU and a TI dose of 40U with a dose ratio of 5. For these doses the onset of action of TI was found to be faster, indicated by a 3.5-fold shorter time to reach 10% of the total effect. Also, the duration of action of TI was shorter, as reflected by a 1.6-fold shorter time to reach 90% of the total effect. This faster PD profile would be expected to translate into an immediate postprandial glucose control after a meal.

Conflict of Interest. The clinical study was sponsored by MannKind Corporation. MG and RB are employees of MannKind. DR, TK, RD, RJ, and AB are employees of Sanofi. Technosphere and Afrezza are registered trademarks of MannKind Corporation.

Author Contributions. D.R., R.D., A.B., R.J., and T.K. wrote the article; M.G. and R.B. designed the research; D.R. and T.K. analyzed the data.

1. Morris, A., Boyle, D., McMahon, A., Greene, S., MacDonald, T. & Newton, R. Adherence to insulin treatment, glycaemic control, and ketoacidosis in insulin-dependent diabetes mellitus. The DARTS/MEMO Collaboration. Diabetes Audit and Research in Tayside Scotland. *Medicines Monitoring Unit. Lancet* **350**, 1505–1510 (1997).
2. Leone-Bay, A. & Grant, M. Technosphere/insulin: mimicking endogenous insulin release. In *Modified-Release Drug Delivery Technology* (eds. Rathbone M., et al.) 673–679. New York: Informa Healthcare USA (2008).
3. Boss, A., Petrucci, R. & Lorber, D. Coverage of prandial insulin requirements by means of an ultra-rapid-acting inhaled insulin. *J. Diabetes Sci. Technol.* **6**, 773–779 (2012).
4. Richardson, P. & Boss, A. Technosphere insulin technology. *Diabetes Technol. Ther.* **9**(suppl. 1), S65–S72 (2007).
5. Marino, M.T., Cassidy, J.P., Smutney, C.C., Zupon, M. & Kocinski, J. Bioequivalence and dose proportionality of AFREZZA® inhalation powder administered using a Gen2 inhaler compared to the MedTone® inhaler. *J. Diabetes Sci. Technol.* **5**, 457–A95 (2011).
6. Baughman, R.A., Amin, A., Watkins, E., Chung, P.-C., Nezamis J. & Boss, A.H. Phase 1, open-label, randomized dose proportionality study of technosphere insulin inhalation powder (TI) doses up to 80U administered with the Gen2 inhaler in healthy subjects. *Diabetes* **62**(suppl. 1), A251, 982–P (2013).
7. Rüppel, D. *et al.* A population PK/PD model of technosphere insulin administered to healthy subjects. *Diabetes* **65**(suppl. 1), A252, 975–P (2016).
8. Potocka, E., Baughman, R.A. & Derendorf, H. Population pharmacokinetic model of human insulin following different routes of administration. *J. Clin. Pharmacol.* **51**, 1015–1024 (2011).
9. Landersdorfer, C. & Jusko, W. Pharmacokinetic/pharmacodynamic modelling of glucose clamp effects of inhaled and subcutaneous insulin in healthy volunteers and diabetic patients. *Drug Metab. Pharmacokinet.* **25**, 418–429 (2010).

10. Potocka, E., Baughman, R., Derendorf H. A population PK/PD model of technosphere insulin administered to healthy and type 2 diabetic subjects. *Diabetes* **59**(suppl. 1), 624–P (2010).
11. Bode, B.W., McGill, J.B., Gross, J.L., Chang, P.C., Bregman, D.B.; Affinity 1 Study Group. Inhaled technosphere insulin compared with injected prandial insulin in type 1 diabetes: a randomized 24-week trial. *Diabetes Care* **38**, 2266–2273 (2015).
12. Baughman, R. *et al*. Technosphere insulin powder (TI) displays earlier onset and shorter duration than insulin Lispro (Lispro). *Diabetes* **65**(suppl. 1 A), 100–LB (2016).
13. Becker, R.H., Frick, A.D., Nosek, L., Heinemann, L. & Rave, K. Dose-response relationship of insulin glulisine in subjects with type 1 diabetes. *Diabetes Care* **30**, 2506–2507 (2007).

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