

ORIGINAL ARTICLE

Implications for Drug Characterization in Glucose Tolerance Tests Without Insulin: Simulation Study of Power and Predictions Using Model-Based Analysis

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In antihyperglycemic drug development, drug effects are usually characterized using glucose provocations. Analyzing provocation data using pharmacometrics has shown powerful, enabling small studies. In preclinical drug development, high power is attractive due to the experiment sizes; however, insulin is not always available, which potentially impacts power and predictive performance. This simulation study was performed to investigate the implications of performing model-based drug characterization without insulin. The integrated glucose-insulin model was used to simulate and re-estimated oral glucose tolerance tests using a crossover design of placebo and study compound. Drug effects were implemented on seven different mechanisms of action (MOA); one by one or in two-drug combinations. This study showed that exclusion of insulin may severely reduce the power to distinguish the correct from competing drug effect, and to detect a primary or secondary drug effect, however, it did not affect the predictive performance of the model.

CPT Pharmacometrics Syst. Pharmacol. (2017) 6, 686–694; doi:10.1002/psp4.12214; published online 25 September 2017.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The power of drug characterization with pharmacometric analysis has been shown to be high. In antihyperglycemic drug development, pharmacometric analysis has successfully been used to characterize various drug effects and semimechanistic models are becoming used more in translation from preclinical experiments to clinical trials. Preclinical experiments are commonly small, thus, pharmacometric analysis is attractive. However, insulin is not always available and the impact of missing a biomarker for the analysis is unknown and may affect both the power and predictive performance, as the high power of pharmacometric analysis is related to the utility of multiple biomarkers.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The implications of performing a model-based analysis with an integrated glucose-insulin model without

insulin, both in terms of power to detect drug effect and predictive properties were answered.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ Performing a pharmacometric analysis using the integrated glucose-insulin model without insulin may severely reduce the power to discriminate the correct from the incorrect drug effects and detect a primary or a secondary drug effect, however, the predictive performance of the model was not affected.

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

☑ The power for drug characterization, with a pharmacometrics analysis, may be severely reduced if insulin is not available and, although the predictive performance is unaffected, the model-building for translation of drug effect from small preclinical experiments to clinical trials may be affected as there is a risk of missing an actual drug effect or selecting an erroneous mechanism of action.

In early hyperglycemic drug development, glucose provocation studies are usually performed to characterize the drug and learn about the mechanism of action (MOA). These glucose challenges are typically performed after a single dose of study drug/placebo or a short induction phase (e.g., 7 days). After a period of fasting, blood sampling is started with fasting blood sample(s), followed by glucose administration, and then blood samples are taken periodically (for example, every 30 minutes for 3–8 hours).^{1–5} These samples are analyzed with regard to glucose and insulin to generate dynamic profiles in the absence and presence of the study compound. Preclinically, the glucose

protocols differ slightly from other glucose administrations (e.g., intraperitoneal), different duration of, or no, fasting prior to glucose challenge, but, most importantly, sometimes only measuring glucose.^{6–8}

Pharmacometric analysis based on time-course data is increasingly used in drug development, due to its integrative nature and the ease with which it can handle dynamic relationships.^{9–11} There are several examples in which pharmacometric analysis has been shown to be highly powerful in phase II trials.^{9,10} The high study power with pharmacometric analysis is most probably achieved by the simultaneous analysis of all subjects'

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Received 7 March 2017; accepted 18 May 2017; published online on 25 September 2017. doi:10.1002/psp4.12214

Table 1 Value of parameters used in simulation and analysis

		Typical value	Interindividual variability
Glucose			
V_G , L	Central volume of distribution	9.33 ^a	30 ^b
V_P , L	Peripheral volume of distribution	8.56 ^a	30 ^b
CL_G , L/min	Insulin-independent clearance	0.0287	59
CL_{GI} , L/min	Insulin-dependent clearance	0.0059	46
Q , L/min	Intercompartmental clearance	0.442 ^a	85 ^b
K_{GE} , min ⁻¹	Effect delay rate constant, glucose on insulin secretion	0.0289 ^a	8 ^b
IPRG	Shape and magnitude of effect, glucose on insulin secretion	1.42 ^a	35 ^b
BIO_G , %	Bioavailability	0.811 ^a	-
K_{ABS} , min ⁻¹	Absorption rate constant	0.0364 ^a	19 ^b
$INCR_{max}$	Maximal incretin effect	1.47 ^a	55 ^b
$INCR_{50}$	Absorbed glucose at 50% incretin	14.8 ^a	114 ^b
G_{ss} , mg/dL	Baseline glucose concentration	169	14
Insulin			
V_I , L	Volume of distribution	6.09 ^a	41 ^b
CL_I , L/min	Insulin clearance	1.22 ^c	29 ^c
K_{IE} , min ⁻¹	Effect delay rate constant, insulin on CL_{GI}	0.0213 ^a	58 ^b
I_{ss} , mU/L	Baseline insulin secretion	8.71 ^c	49 ^c
Drug			
K_{aD} , min ⁻¹	Rate constant, drug absorption	0.0365 ^a	68 ^a
CL_D , L/min	Drug clearance	0.682 ^a	15 ^a
V_D , L	Volume of distribution, drug	155 ^a	29.6 ^a
EC_{50D}	Drug effect at 50% E_{max}	0.1	30
$E_{maxD(incr)}$	Maximal drug effect, INCR activity	1.0	-
$E_{maxD(BINS)}$	Maximal drug effect, basal insulin secretion	0.5	-
$E_{maxD(CLG)}$	Maximal drug effect, CLG	2.0	-
$E_{maxD(CLGI)}$	Maximal drug effect, CLGI	0.5	-
$E_{maxD(EGP)}$	Maximal drug effect, glucose production	0.7	-
$E_{maxD(GABS)}$	Maximal drug effect, glucose absorption	0.02	-
$E_{maxD(GSEN)}$	Maximal drug effect, glucose sensitivity	1.5	-
Residual error			
RESG, %	Residual error, glucose	0.0732	-
RESI, %	Residual error, insulin	0.252	-
Parameter correlations			
$Corr_{VG-Q}$	Correlation between VG and Q	-0.19	-
$Corr_{VG-VI}$	Correlation between VG and VI	0.7225	-
$Corr_{Q-VI}$	Correlation between Q and VI	-0.122	-
$Corr_{CLD-VD}$	Correlation between CLD and VD	0.0311	-

^aFixed for analysis. ^b Fixed to zero for analysis. ^c Fixed for analysis without insulin measurements.

longitudinal measurements and integration of several biomarkers.¹²

Although mainly used in clinical drug development, pharmacometric analysis is also becoming used more frequently in preclinical drug development. Preclinical experiments are commonly performed in few animals, thus, the high power of pharmacometric analyses is attractive. In antihyperglycemic drug development, protocol differences can be handled using pharmacometric analysis and integrating several biomarkers in a semimechanistic manner enables preclinical to clinical translation. However, the lack of the insulin could potentially impact the power and predictive performance significantly and, thus, the benefits of a pharmacometric analysis in the absence of insulin may not be as great as expected. To this end, the impact of missing insulin in a pharmacometric

analysis with an integrated glucose-insulin model was investigated in this simulation study for seven drug MOAs. The investigations were divided into four parts: (1) power to identifying a drug effect; (2) power to distinguish the correct MOA from competing incorrect MOAs; (3) power to identifying the secondary drug MOA in addition to the primary MOA; and (4) the impact on glucose predictions in terms of accuracy and precision with or without insulin.

METHODS

Study design

The design used in this simulation study resembled the design used by Jauslin *et al.*² in which an oral glucose tolerance test (OGTT) was used in early drug development. A

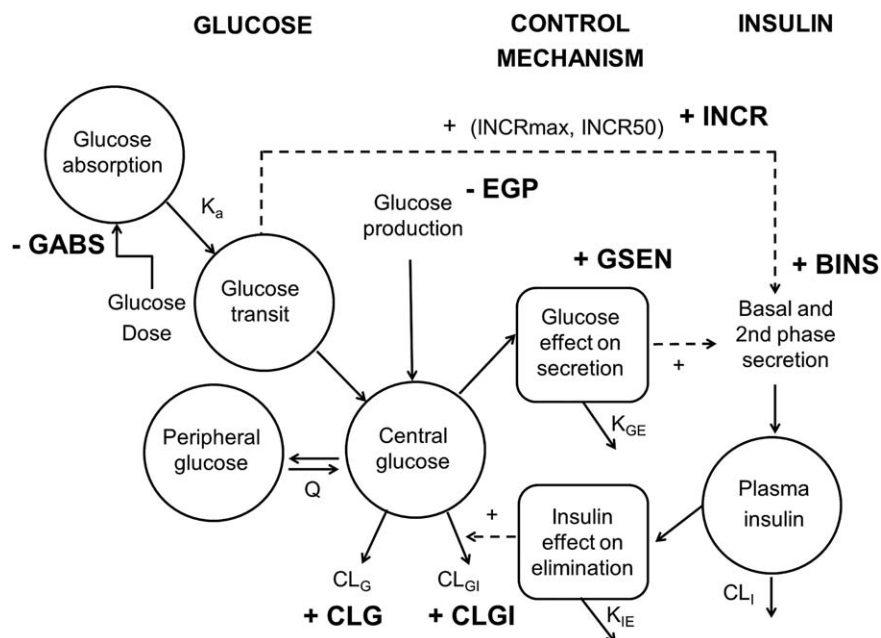


Figure 1 Schematic presentation of integrated glucose-insulin oral glucose tolerance test model in type 2 diabetes mellitus (Jauslin *et al.*²) with seven drug mechanism of actions. BINS, basal insulin secretion; CL_G, insulin-independent glucose clearance; CL_{GI}, insulin-dependent glucose clearance; CL_I, insulin clearance; EGP, endogenous glucose production; GABS, glucose absorption; GSEN, glucose sensitivity; INCR, incretin activity.

crossover design was used with two arms: placebo and 50 mg of the study compound. At either occasion, the placebo or study compound was administered at time 0, followed by the administration of oral glucose (75 g) at time 30 minutes. Blood samples were taken at times 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes.

Simulation of data

Datasets with glucose and insulin and only glucose were simulated according to the above described design using the integrated glucose-insulin (IGI) model for OGTT with parameters as reported by Jauslin *et al.*² (listed in **Table 1**). The model, developed by Jauslin *et al.*² was chosen because it is a closed-loop system, enabling integrated simulations of glucose and insulin, and it has shown good estimation and prediction performance as well as the ability to characterize drug effects.^{3,13–16}

The pharmacokinetics (PKs) of the study compound was described using an oral one-compartment model with first order elimination, parameterized in terms of absorption rate constant, clearance (CL_D), and volume of distribution (V_D). The PK parameters were chosen such that the time of maximum plasma concentration (T_{max}) was observed after 1 hour and the 4-hour postdose concentration was half of peak plasma concentration (C_{max}), ensuring a compound eligible for twice daily dosing (b.i.d.; see **Table 1**). The parameters chosen resulted in C_{max} around 0.243 mg/L at 65 minutes and a 4-hour concentration of 0.127 mg/L after a dose of 50 mg. The drug concentrations were used in simulating glucose and insulin in the presence of the drug; however, it was not used in the analysis, where PK was kept fixed.

Seven drug MOAs were investigated: stimulation of (1) incretin (INCR); (2) basal insulin secretion (BINS); (3) insulin-independent glucose clearance (CL_G); (4) insulin-dependent glucose clearance (CL_{GI}) and (5) glucose sensitivity (GSEN), as well as inhibition of (6) endogenous glucose production (EGP) and glucose absorption (GABS). **Figure 1** illustrates the IGI-OGTT model with MOAs and **Supplementary Figure S1** shows the typical profiles of glucose and insulin in relation to placebo. Each of the investigated MOAs correspond to the expected primary MOAs of drugs or compounds: dipeptidyl peptidase-4 inhibitors (DPP-4i)¹⁷ prolonging the action of INCR hormones; sulfonylureas¹⁸ increasing insulin secretion; sodium-glucose cotransporter-2 inhibitors (SLGT2i)¹⁹ increasing insulin-independent glucose elimination; peroxisome proliferator-activated receptor agonists²⁰ increasing insulin sensitivity; metformin²¹ decreasing EGP; α -glucosidase inhibitors²² decreasing glucose absorption; and G-protein-coupled receptor 40 (GPR40) agonists²³ increasing glucose-stimulated insulin secretion.

The pharmacodynamics (PDs) were simulated with the concentration of drug giving half-maximal effective concentration (EC₅₀) set to 0.1 mg/L, which the study compound concentrations exceeded 10 minutes after the administration and throughout the experiment. The maximum effect E_{maxD}, was titrated for each MOA to produce the same effect: 10% reduction in glucose area under the curve (AUCG). Glucose exposure (i.e., AUCG) is the main driver of HbA1c formation. However, HbA1c, as measured with the National Glycohemoglobin Standardization Program assay, also contains an additive, nonglucose-related binding of 2.15%.²⁴ Thus, a 10% reduction of glucose exposure corresponds to an approximate reduction in HbA1c from

8.7% to 8.0%, which corresponds with the minimum required drug effects of a compound eligible as an antihyperglycemic drug.²⁵ All the parameter values are summarized in **Table 1**.

For part 1 (identify a drug effect) and part 2 (distinguishing the correct MOA), data were simulated using one MOA at a time of the seven MOAs. In part 3 (identifying a secondary drug MOA on top of the primary), two MOAs were combined in the simulations. The drug effects in part 3 were simulated as independent of each other and only a selection of combinations was simulated. The selected combinations of MOAs were inspired by the reported secondary effects of DDP-4is, sulfonylureas, SLGT2is, PPAR agonists, metformin, and GPR40 agonists. Secondary effects of GPR40 agonists are sparsely reported and, thus, this MOA was arbitrarily investigated together with increasing CL_G (peripheral glucose disposal) and decreasing EGP, as well as increased CL_{GI} as insulin increases by the primary effect. No secondary effects were investigated with GABS, as the effect of α -glucosidase inhibitors is local in the gastrointestinal tract with no systemic effects. The detail information on the study setup is summarized **Supplementary Table S1**.

Study power

Analysis of data, both with and without insulin, was performed using the IGI model with many parameters fixed and greatly reduced interindividual variability (IIV). When analyzing with insulin, the estimated parameters were CL_G and CL_{GI} , insulin clearance (CL_I), baseline glucose (G_{SS}), baseline insulin, E_{maxD} , and EC_{50D} , with IIV on all estimated parameters, except for the E_{maxD} . All remaining parameters of the IGI model were fixed to the published values with removed IIVs. In the absence of insulin, baseline insulin and CL_I with associated IIVs were fixed to the published values.

A full and a reduced model were fitted to the data to assess the power for the drug MOAs, as shown in **Figure 1** and listed in **Supplementary Table S1**. The NONMEM code for the implementation of the drug effects can be found in **Supplementary Table S1**. In part 1, the full model contained the correct drug MOA, whereas the reduced model contained no drug effect. In part 2, the full (i.e., correct model) contained the drug effect at the correct MOA (as in part 1) with the reduced (i.e., competing) model containing the drug effect at any of the incorrect MOAs. In part 3, the full model contained the two correct MOAs and the reduced model contained only the correct primary MOA.

The calculation of study power of part 1 and part 3 was done using the likelihood ratio test (LRT) with the chosen significance level (α) of 5%, and the degree of freedom (df) set to the number of differing parameters between the competing models. The critical value, according to the LRT, was 7.81, at $\alpha = 5\%$ with $df = 3$. For part 2, where the competing models were nonhierarchical, the LRT could not be used. To determine superiority between the competing models, an arbitrary critical value of 10 was used (i.e., the difference in delta objective function value (ΔOFV) had to be larger than 10 in favor for the correct model to be

deemed superior, or else the competing models were deemed to be of similar quality).

The difference in study power between analyses with and without insulin was assessed using the relative power (i.e., ratio of the ΔOFV for analysis with and without), denoted $\Delta OFV_{gi/g}$.

$$\Delta OFV_{gi/g} = \frac{OFV_{full,gi} - OFV_{red,gi}}{OFV_{full,g} - OFV_{red,g}} \quad (1)$$

In which $OFV_{full,gi}$ and $OFV_{full,g}$ are OFVs of full models, including and excluding insulin, respectively, and $OFV_{red,gi}$ and $OFV_{red,g}$ are OFVs of reduced models, including and excluding insulin, respectively. The df will be the same for the nominator and denominator as the only parameters differing between the full and reduced models are the drug effect parameters. The ratio reflects the fraction of subjects needed to achieve the same study power when analyzing with and without insulin (i.e., $\Delta OFV_{gi/g} = 2.0$ means that twice as many subjects are needed to achieve an equal power when analyzing without insulin compared to with insulin). The relative power was assessed with simulations of 500 subjects.

To support the interpretation of the $\Delta OFV_{gi/g}$, the Monte Carlo Mapped Power (MCMP) method⁹ was used in addition to the $\Delta OFV_{gi/g}$ in part 1 to assess the number of subjects needed for 95% power at $\alpha = 5\%$, 1%, and 0.1%.

Accuracy and precision

In part 4, estimation of accuracy and precision was performed for AUCG and assessed as the ratio with and without the drug:

$$AUCG_{D/PL} = \frac{AUCG_D}{AUCG_{PL}} \quad (2)$$

The true $AUCG_{D/PL,true}$'s were determined from 15 subjects simulated 500 times using the parameter estimates of **Table 1** (simulation values). These $AUCG_{D/PL,true}$'s are expected to be around 0.9, as set by PK and PD simulation parameters. These 500 datasets were estimated with the full models, including and excluding of insulin data. Each of the 2×500 sets of estimates were then used to simulate datasets with 15 subjects, producing 500 $AUCG_{D/PL,gi}$ (including insulin) and 500 $AUCG_{D/PL,g}$ (excluding insulin). The relative estimation errors (REEs) with and without insulin were calculated as shown in the Eqs. 3a and 3b.

$$REE(AUCG_{gi}) = \frac{AUCG_{D/PL,gi} - AUCG_{D/PL,true}}{AUCG_{D/PL,true}} \quad (3a)$$

$$REE(AUCG_g) = \frac{AUCG_{D/PL,g} - AUCG_{D/PL,true}}{AUCG_{D/PL,true}} \quad (3b)$$

Software

Simulation and estimation of data were done using nonlinear mixed-effects modeling with first-order conditional estimation method in NONMEM version 7.3²⁶ with the differential equation solver ADVAN13. Datasets for NONMEM and all graphs were prepared and produced using

Table 2 Part 1: the relative study power to detect a drug effect, presented as ratio of individuals needed for the same power with and without insulin

Assessed with $\Delta OFV_{gi/g}$	INCR	BINS	CL _G	CL _{GI}	EGP	GABS	GSEN
Ratio of subjects (with/without insulin)	1.5	1.5	1.2	1.2	1.2	1.3	2.1
Assessed with MCMP at 95% power							
No of subjects, $\alpha = 5\%$ with insulin	4	4	10	6	11	5	<3
No of subjects, $\alpha = 5\%$ without insulin	5	6	13	7	14	6	4
Ratio of subjects (with/without insulin), $\alpha = 5\%$	1.3	1.5	1.3	1.2	1.3	1.2	>1.3
No of subjects, $\alpha = 1\%$ with insulin	4	5	11	7	12	5	<3
No of subjects, $\alpha = 1\%$ without insulin	6	7	15	8	14	6	5
Ratio of subjects (with/without insulin), $\alpha = 1\%$	1.5	1.4	1.4	1.1	1.2	1.2	>1.7
No of subjects, $\alpha = 0.1\%$ with insulin	5	6	13	9	15	7	<3
No of subjects, $\alpha = 0.1\%$ without insulin	7	8	17	10	17	9	6
Ratio of subjects (with/without insulin), $\alpha = 0.1\%$	1.5	1.3	1.3	1.1	1.1	1.3	>2.0

The table lists the ratio of ΔOFV for the analysis with insulin relative to without insulin, $\Delta OFV_{gi/g}$ as well as the number of subjects needed to achieve a power of 95% at 5%, 1%, and 0.1% significance levels for all investigated drug effect MOAs.

BINS, basal insulin secretion; CL_G, insulin-independent glucose clearance; CL_{GI}, insulin-dependent glucose clearance; ΔOFV , delta objective function value; EGP, endogenous glucose production; GABS, glucose absorption; GSEN, glucose sensitivity; INCR, incretin activity; MCMP, Monte Carlo Mapped Power.

R²⁷ with the ggplot2 package.²⁸ The SSE^{29,30} and MCMP,³¹ as implemented in PsN, were used for data simulation, power estimation, and estimations of AUCG. The calculation of REE was done using R.²⁷

RESULTS

Part 1: power to identify a drug effect

As shown in **Table 2**, the power to detect a drug effect was higher when including insulin for all drug-investigated MOAs. The largest difference in power was observed for detecting the drug effect on glucose sensitivity, $\Delta OFV_{gi/g} = 2.1$. This was followed by equal improvement for incretin effect and insulin secretion, thereafter glucose absorption, and the lowest improvement for glucose clearance and EGP.

The relative power, $\Delta OFV_{gi/g}$, assessed using the number of subjects from the MCMP method and the ΔOFV , gave similar results (**Table 2**). For a drug effect on glucose sensitivity, the study power was extremely high when including insulin; 99% study power was achieved with three subjects at $\alpha = 0.1\%$, thus, the granularity to assess the ratio of subjects was too small. In general, a high power (95%) was achieved with few subjects when using pharmacometrics model-based analysis.

Part 2: power to distinguishing the correct MOA from an incorrect

As seen in **Table 3**, the power to identify the correct MOA effects was largely affected by the exclusion of insulin for most scenarios. The most pronounced was the difference in power for identifying correctly the MOAs on CL_{GI} when competing with incretin effect and secretion. In these cases, >100 subjects were needed when insulin was lacking, indicating perhaps a nonidentifiability of drug effect without insulin. In addition, more than four times the sample size was needed when analyzing without insulin compared to with insulin to achieve equal power in distinguishing drug MOAs of INCR from insulin secretion and insulin-dependent glucose clearance, insulin secretion from insulin-dependent glucose clearance, insulin-independent glucose clearance from insulin secretion, and glucose sensitivity from incretin effect

and insulin secretion. For most of other drug MOAs, at least 1.5 times fewer subjects were needed to distinguish correct from incorrect MOAs with the inclusion of insulin. However distinguishing insulin-dependent glucose clearance from insulin-independent glucose clearance, EGP and glucose absorption, or distinguishing EGP from glucose absorption was not influenced by exclusion of insulin. Furthermore, the insulin-independent glucose clearance could not be discriminated from EGP even when including insulin.

Part 3: power to identify the secondary drug MOA on top of the primary MOA

As shown in **Table 4**, the overall power to detect secondary drug MOAs was higher when including insulin and most pronounced for detecting insulin secretion on top of incretin effect and insulin-dependent glucose clearance on top of insulin secretion. In these cases, >100 subjects were needed to detect the secondary drug MOAs on top of the

Table 3 Part 2: the relative study power to distinguish correct drug mechanism of action from incorrect represented as ratio of subjects needed for same power without and with insulin

Correct MOA	Competing incorrect MOA						
	INCR	BINS	CL _G	CL _{GI}	EGP	GABS	GSEN
INCR	-	4.7	1.7	4.1	2.5	2.5	1.6
BINS	^a	-	1.8	6.0	2.5	3.1	2.0
CL _G	3.8	4.7	-	1.5	^b	1.6	2.4
CL _{GI}	^a	^a	1.0	-	1.0	1.0	2.5
EGP	2.9	3.1	1.0	1.4	-	1.1	1.6
GABS	3.7	3.8	1.2	1.4	1.2	-	2.3
GSEN	4.4	4.5	2.5	3.5	2.6	3.1	-

The table lists the relative power based on ΔOFV for the analysis with and without insulin, $\Delta OFV_{gi/g}$, thus $\Delta OFV_{gi/g} = 2$ means twice as many subjects are needed in a study in which insulin is excluded to attain the same power as for a study including insulin.

BINS, basal insulin secretion; CL_G, insulin-independent glucose clearance; CL_{GI}, insulin-dependent glucose clearance; ΔOFV , delta objective function value; EGP, endogenous glucose production; GABS, glucose absorption; GSEN, glucose sensitivity; INCR, incretin activity; MOA, mechanism of action.

^aMore than 100 subjects needed to distinguish the drug effect without insulin. ^b >100 subjects needed to distinguish the drug effect independent of inclusion or exclusion of insulin.

Table 4 Part 3: the relative study power to detect correct secondary correct drug effect with the primary already included, represented as ratio of subjects needed for same power without and with insulin

Secondary MOA	True primary MOA					
	INCR	BINS	CL _G	CL _{GI}	EGP	GSEN
BINS	^a	-	-	-	-	-
CL _G	-	1.1	-	1.4	^b	-
CL _{GI}	-	^a	-	-	1.1	3.7
EGP	1.6	2.1	1.0	1.0	-	1.8
GABS	8.1	-	1.5	-	-	-
GSEN	4.3	6.0	-	4.9	4.9	-

The table lists the ratio of Δ OFV for the analysis with insulin relative to without insulin, Δ OFV_{gi/g}.

BINS, basal insulin secretion; CL_G, insulin-independent glucose clearance; CL_{GI}, insulin-dependent glucose clearance; Δ OFV, delta objective function value; EGP, endogenous glucose production; GABS, glucose absorption; GSEN, glucose sensitivity; INCR, incretin activity; MOA, mechanism of action.

^aMore than 100 subjects needed to distinguish the drug effect without insulin. ^b>100 subjects needed to distinguish the drug effect with and without insulin.

primary when insulin was missing. In addition, when insulin was lacking, at least twice as many subjects were needed to detect insulin-dependent glucose clearance on top of glucose sensitivity, EGP on top of insulin secretion, glucose absorption on top of incretin effect, and glucose sensitivity on top of all tested primary MOAs. A slight increase of power to detect the secondary MOAs was observed from insulin-independent on top of insulin-dependent glucose clearance, EGP on top of incretin effect, and glucose sensitivity, as well as glucose absorption on top of insulin-independent glucose clearance. However, there was no difference in power to detect insulin-independent glucose clearance on top of EGP, and EGP on top of glucose clearance. Moreover, in identifying a secondary drug MOA on insulin-independent glucose clearance on top of primary MOA on EGP, >100 subjects were required, independent of exclusion or inclusion of insulin.

Part 4: accuracy and precision of glucose predictions

The AUC_{D/PL} (see **Figure 2**) was found to be estimated with an adequate accuracy to the true simulation value and with good precision, both including and excluding insulin data in the analysis. Hence, the prediction of glucose was not affected by insulin exclusion.

DISCUSSION

High-powered study designs enable characterization of drug effects with a smaller number of subjects (humans or animals). A high-power study is also efficient, undeniably reducing the time and cost spent in the development of new drugs.¹⁰ The aim of this study was to investigate the impact of omitting insulin on power to identify a drug effect, distinguish the primary drug effect, identify the secondary drug effect, and predicting glucose with a model-based analysis.

Several factors affect the study power: the number of studied subjects and samples; the size of the drug effect;

and the variability in data. In an attempt to control these factors, relative power was used as opposed to absolute. Relative power assessed the ratio of subjects needed to achieve the same power. Thus, this metric is independent on the number of subjects. Additionally, using relative power constrained the influence of data variability to insulin variability, as the nominator and denominator of the relative power cancels out glucose variability. Furthermore, by reducing IIV in the model for the analysis and not the simulations, unaccounted IIV resulted in increased residual error, which reduces the benefit of including insulin measurements. The drug effect size was chosen based on the minimum requirements for an oral antihyperglycemic drug in terms of PK and PD. Selecting PK parameters such that absorption was fast and elimination was as fast as acceptable for b.i.d. administration resulted in large fluctuations in concentrations. The larger the fluctuations were in PK the smaller the benefit of insulin, due to the increased variability. In addition, PD was titrated to 10% AUCG reduction, which, as described in the Methods section, is the minimal requirement for a compound eligible for antihyperglycemic treatment. A higher PD effect would result in larger benefits of including insulin in the analysis. Reducing the number of samples of glucose/insulin would reduce the relative power, as there would be a smaller difference between inclusion and exclusion of insulin. However, the sampling design used in this project represents a commonly used design in early clinical drug development.

The study power in this work was generally high, as seen from **Table 2**. For 95% power to detect a drug effect, with $\alpha = 5\%$, a minimum of 3 and 4 (maximum 11 and 14) subjects were needed, with and without insulin, respectively. For distinguishing the correct MOA and detecting a secondary effect, an average of twice as many subjects were needed. As the high power of pharmacometric analysis is related to the greater number of samples and more biomarkers utilized, it is unsurprising that power is lower if insulin is not available. The IGI model used in the current work integrates glucose and insulin in the model; however, there are versions of the model, including other biomarkers in diabetes (e.g., c-peptide³² and glucagon¹³). The results would most likely be similar if insulin was replaced by c-peptide, as these biomarkers carry similar information. However, the impact of the exclusion of other biomarkers (e.g., glucagon) would largely depend on what information the biomarker contributes. Although the power would most likely be lower when the biomarker is missing.

For a model-based simulation study of glucose and insulin, such as the current study, the choice of the model is limited to the closed-loop systems. This disqualifies models such as Bergman's minimal model,³³ in which insulin observations drive glucose. Bergman's minimal model, as opposed to the IGI model, expresses insulin sensitivity and glucose effectiveness as explicit parameters, which is attractive. The simulated MOAs are limited to explicitly implemented parameters and, thus, drug effects (e.g., glucose effectiveness and insulin sensitivity) were assumed to affect CL_G and CL_{GI} in the IGI model. If a particular parameter of the glucose homeostasis is of interest, the approach presented in this work could be repeated using a closed-

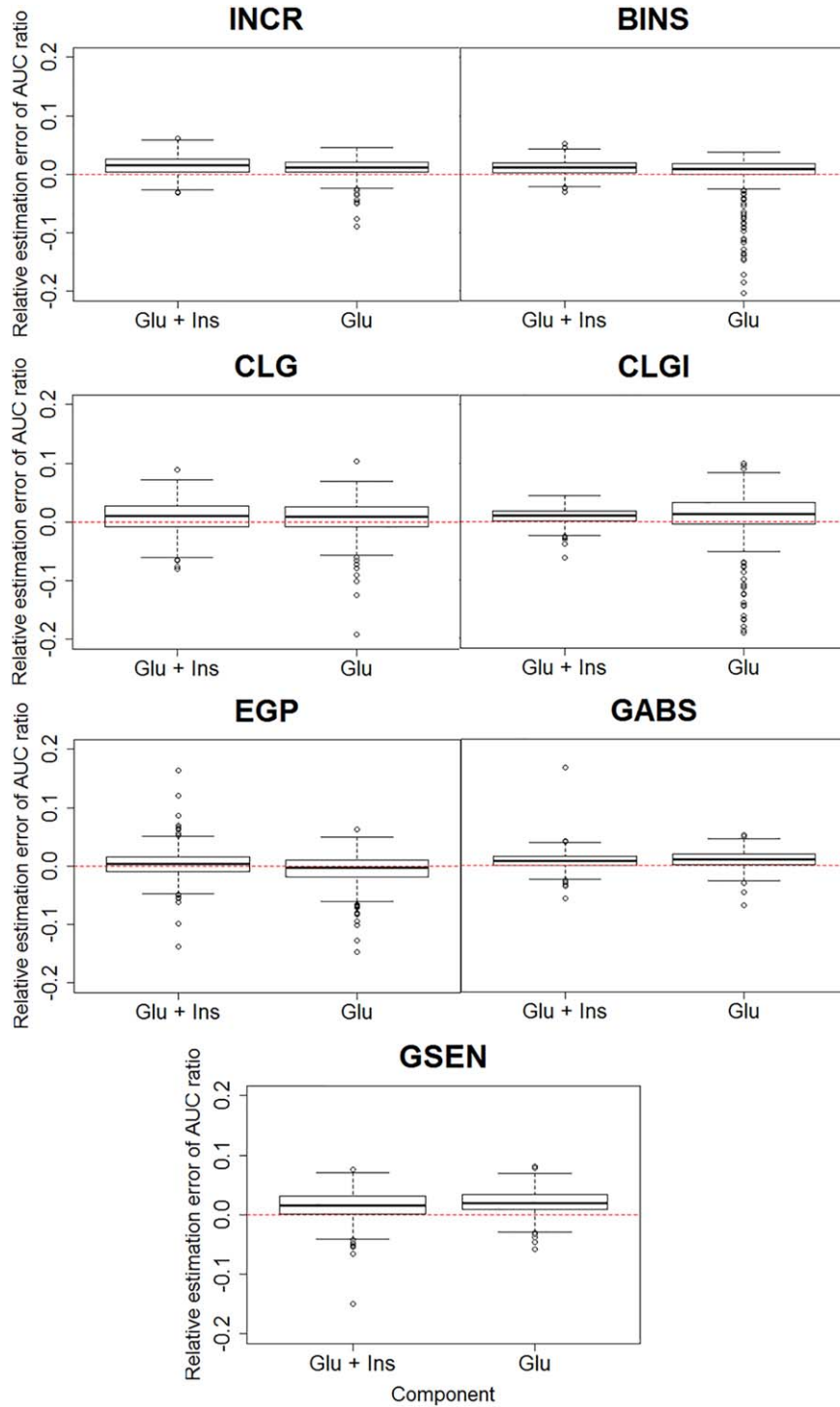


Figure 2 Part 1: relative estimation error for glucose under the curve (AUCG) ratio in seven drug effects. BINS, basal insulin secretion; CLG, insulin-independent glucose clearance; CLGI, insulin-dependent glucose clearance; EGP, endogenous glucose production; GABS, glucose absorption; Glu, glucose only data; Glu + Ins, glucose and insulin data; GSEN, glucose sensitivity; INCR, incretin activity.

loop system model in which the parameters of interest are explicitly implemented.

An OGTT design was chosen in this work, although the meal tolerance test (MTT) might be a better tolerated

design, triggering a more physiological response. The translation of these results to an MTT is related to the differences in the IGI model for these glucose challenges. In the IGI model, the main differences between an OGTT and

an MTT are the rate of glucose absorption, being slower in MTT than OGTT and the incretin effect, being higher in MTT than in OGTT. The rate of glucose absorption and INCR effect will work in opposite directions; with the incretin increasing insulin secretion, whereas a slower rate of glucose absorption will give lower glucose peak, resulting in a lower insulin secretion. As the incretin effect is larger than the effect of the absorption rate, the net effect is higher insulin in the MTT. Thus, the relative power is expected to be slightly higher, benefiting inclusion of insulin. In addition, other glucose challenges than OGTT and MTT are used in drug development (e.g., graded glucose infusion and clamps). Especially in preclinical drug development are the challenges different and investigating, therefore, which challenge design is most powerful for different drug effect MOAs is, thus, an interesting extension of this work.

The current investigation in part 3 does not fully cover all combinations, but it serves as an example of combinations as well as describes a methodology to investigate more combinations. Moreover, as the secondary drug effects were well detected and differentiated from the primary in this study, we could expect that combination therapy with two combined agents could also be well, or even better, characterized, as differences also in PK between the two drugs would improve the power to separate effects.

The inclusion of insulin added power to both detecting a drug effect and distinguishing the correct drug MOA from the incorrect drug MOA in most cases. As expected, the increase in power with inclusion of insulin was largest when MOAs were related to insulin (BINS and INCR), and smaller when the MOAs were related to the glucose (CL_G , EGP, and GABS). The CL_{GI} had little or no gain in power of including insulin when tested against the glucose-related parameters. At baseline (fasting conditions), CL_{GI} and CL_G contribute equally to the elimination of glucose. As insulin increases due to the glucose challenge, the relative importance of CL_{GI} to CL_G increases. A large difference is seen in the dynamic glucose profile of a drug effect on CL_{GI} , although this is not seen for CL_G . Thus, insulin observations are not needed to the same extent to detect the difference between MOA on CL_{GI} vs. CL_G . The same reasoning applies for EGP. Consistently, when discriminating CL_{GI} from insulin secretion either as a competing MOA or as a secondary effect, insulin measurements gave a large improvement in power. A similar trend was also seen with CL_{GI} and INCR. This behavior could be explained by the nature of the parameter with CL_{GI} being related to both glucose and insulin. GSEN, which also is related to both glucose and insulin, unlike CL_{GI} , behaved much more as a pure insulin-related parameter, with a large power increase with the inclusion of insulin measurements in the analysis.

To distinguish a correct MOA on CL_G from an incorrect MOA on EGP was not feasible with the settings of this project. Both with and without insulin, there were >100 subjects needed in a study. This is not surprising as the CL_G and EGP (also insulin-independent) are two sides of the same coin. A decrease of insulin-independent input of glucose is indistinguishable from an increase of insulin-independent output of glucose, without tracer glucose in the design.

Parameter accuracy and precision were assessed using the mean ratio of $AUC_{G_{D/PL}}$, representing magnitude of reduction of glucose AUCG with and without drug treatment. An adequate accuracy and a good precision for $AUC_{G_{D/PL}}$ were achieved in all MOAs, with and without insulin reflecting a good model performance for the estimation of $AUC_{G_{D/PL}}$ and, thus, it seems exclusion of insulin had no impact on precision of $AUC_{G_{D/PL}}$. Thus, if the main focus of a pharmacometric analysis is to quantify the difference in glucose for treatment, insulin is not crucial.

The implications of the results presented in this work are that, although the glucose dynamics can accurately and precisely be predicted, absence of insulin will for most MOAs lead to more subjects being needed to achieve an adequate power for identifying drug effects. This may not be an issue in clinical drug development as the number of subjects in clinical studies usually is sufficiently high for a high power and insulin is almost always measured. However, as mentioned in the introduction, pharmacometric analysis gains popularity preclinically for model development with the aim of translating drug effects into humans. In developing a model fit for translation using animal data, the ability to correctly identify a drug effect is important, but the number of animals in the experiments are often low; not seldom, lower than what is needed for sufficient power,⁶⁻⁸ thus affecting the model building as there is a risk of missing identifying drug effect, primary or secondary, and identifying an erroneous MOA.

CONCLUSION

In conclusion, the power to detect a drug effect was harmed when insulin was not included for all investigated MOAs. The power to distinguish a correct mechanism of a drug MOA from an incorrect mechanism and to detect a secondary on top of a primary MOA, was, in most cases, severely harmed when insulin was missing in the model-based analysis. The $AUC_{G_{D/PL}}$ estimation was accurate and precise in all drug MOAs, independently of whether insulin was used or not. In general, performing a model-based analysis using the IGI model without insulin will affect the power to detect the correct drug MOAs; however, this will not impact the estimates of drug effect in terms of $AUC_{G_{D/PL}}$.

Acknowledgments. The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement number 115156, resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind contribution. The DDMoRe project is also financially supported by contributions from Academic and SME partners.

Conflict of Interest. The authors declared no conflict of interest.

Author Contributions. S.M.S.G., M.C.K., and M.O.K. wrote the manuscript. S.M.S.G., M.C.K., and M.O.K. designed the research. S.M.S.G. performed the research. S.M.S.G., M.C.K., and M.O.K. analyzed the data.

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