Pharmacometrics Modelling in Type 2 Diabetes Mellitus

Implications on Study Design and Diabetes Disease Progression

SITI MAISHARAH SHEIKH GHADZI
Abstract


Pharmacometric modelling is widely used in many aspects related to type 2 diabetes mellitus (T2DM), for instance in the anti-diabetes drug development, and in quantifying the disease progression of T2DM.

The aim of this thesis were to improve the design of early phase anti-diabetes drug development studies with the focus on the power to identify mechanism of drug action (MoA), and to characterize and quantify the progression from prediabetes to overt diabetes, both the natural progression and the progression with diet and exercise interventions, using pharmacometrics modelling.

The appropriateness of a study design depends on the MoAs of the anti-hyperglycaemic drug. Depending on if the focus is power to identify drug effect or accuracy and precision of drug effect, the best design will be different. Using insulin measurements on top of glucose has increase the power to identify a correct drug effect, distinguish a correct MoA from the incorrect, and to identify a secondary MoA in most cases. The accuracy and precision of drug parameter estimates, however, was not affected by insulin. A natural diabetes disease progression model was successfully added in a previously developed model to describe parameter changes of glucose and insulin regulation among impaired glucose tolerance (IGT) subjects, with the quantification of the lifestyle intervention. In this model, the assessment of multiple short-term provocations was combined to predict the long-term disease progression, and offers apart from the assessment of the onset of T2DM also the framework for how to perform similar analysis. Another previously published model was further developed to characterize the weight change in driving the changes in glucose homeostasis in subjects with IGT. This model includes the complex relationship between dropout from study and weight and glucose changes.

This thesis has provided a first written guidance in designing a study for pharmacometrics analysis when characterizing drug effects, for early phase anti-diabetes drug development. The characterisation of the progression from prediabetes to overt diabetes using pharmacometrics modelling was successfully performed. Both the natural progression and the progression with diet and exercise interventions were quantified in this thesis.

Keywords: Pharmacometric, type 2 diabetes mellitus, impaired glucose tolerance, prediabetes, anti-diabetes drug development, insulin, glucose, natural diabetes disease progression, lifestyle intervention, short-term provocation, long-term effect, glucose homeostasis, glucose and insulin regulation, weight, dropout

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urn:nbn:se:uu:diva-317040 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-317040)
To my family:
past, present and future
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I  **Ghadzi SMS**, Karlsson MO, Kjellsson MC. The impact of insulin measurements in oral glucose tolerance test: a simulation study in type 2 diabetes to assess power to characterize drug effects. *Submitted*

II  Ibrahim MMA, **Ghadzi SMS**, Kjellsson MC, Karlsson MO. Study design selection in early clinical anti-diabetic drug development: a simulation study of glucose tolerance tests. *Manuscript*


IV  **Ghadzi SMS**, Karlsson MO, de Mello VD, Uusitupa M, Kjellsson MC, Finnish Diabetes Prevention Study Group. Model-based quantification of the natural diabetes disease progression and lifestyle intervention effects on weight, beta cell function and insulin sensitivity for subjects with impaired glucose tolerance. *Manuscript*

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Contents

Introduction ................................................................................................... 13
  Diabetes mellitus ............................................................................................ 13
    Prediabetes states .......................................................................................... 15
  Disease progression of T2DM ....................................................................... 16
  Non-pharmacological intervention in T2DM .............................................. 18
Antidiabetic drug development in T2DM ..................................................... 19
  Pharmacological treatment of T2DM .......................................................... 19
  Glucose tolerance tests in the antidiabetic drug development ..................... 21
Pharmacometrics ............................................................................................ 23
  Non-linear mixed effect (NLME) models ................................................... 24
  Maximum likelihood estimation method ..................................................... 25
Pharmacometrics modelling in drug development ....................................... 25
Pharmacometrics modelling in T2DM .......................................................... 26

Aims ................................................................................................................ 28

Methods .......................................................................................................... 29
  Data ............................................................................................................... 29
    Simulation from the prior developed model (Paper I & II) ....................... 29
    Finnish Diabetes Prevention Study (FDPS) (Paper III & IV) ................. 31
  Study power calculation (Paper I & II) ....................................................... 31
    ΔOFV ratio between full and reduced model (Paper I & II) ..................... 31
    Monte Carlo Mapped Power (MCMP) Method (Paper I) ....................... 32
  Accuracy and precision of parameter estimates (Paper I & II) ................. 33
    Drug parameters (EmaxD & EC50D) (Paper I) and (θα) (Paper II) ............ 33
    Glucose area under the curve ratio (AUGC_D/AUGC_PL) ....................... 33
  Diabetes disease progression modelling ...................................................... 34
    IGI model (Paper III) .............................................................................. 34
    WHIG model (Paper IV) ....................................................................... 36
  Sensitivity and specificity analyses (Paper III) .......................................... 38
  Logistic regression dropout model (Paper IV) .......................................... 39
  Data analyses and model evaluation .......................................................... 40
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSG&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Absorbed glucose at 50% maximum incretin effect</td>
</tr>
<tr>
<td>AD</td>
<td>Anno domini</td>
</tr>
<tr>
<td>AGI</td>
<td>Alpha-glucosidase inhibitor</td>
</tr>
<tr>
<td>AUGC</td>
<td>Area under the glucose curve</td>
</tr>
<tr>
<td>AUGC&lt;sub&gt;D&lt;/sub&gt;</td>
<td>Area under the glucose curve for drug</td>
</tr>
<tr>
<td>AUGC&lt;sub&gt;PL&lt;/sub&gt;</td>
<td>Area under the glucose curve for placebo</td>
</tr>
<tr>
<td>AUGC&lt;sub&gt;D/PL&lt;/sub&gt;</td>
<td>Ratio of AUGC&lt;sub&gt;D&lt;/sub&gt;/AUGC&lt;sub&gt;PL&lt;/sub&gt;</td>
</tr>
<tr>
<td>AUGC&lt;sub&gt;D/PL,Cx&lt;/sub&gt;</td>
<td>Ratio of AUGC&lt;sub&gt;D&lt;/sub&gt;/AUGC&lt;sub&gt;PL&lt;/sub&gt; for various glucose tests</td>
</tr>
<tr>
<td>B</td>
<td>Beta cell function</td>
</tr>
<tr>
<td>B&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Baseline beta cell function</td>
</tr>
<tr>
<td>BC</td>
<td>Before Christ</td>
</tr>
<tr>
<td>BINS</td>
<td>Basal insulin secretion</td>
</tr>
<tr>
<td>BIOG</td>
<td>Glucose bioavailability</td>
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<tr>
<td>BLWT</td>
<td>Baseline body weight</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CGI</td>
<td>Combined glucose intolerance</td>
</tr>
<tr>
<td>CLG</td>
<td>Insulin-independent glucose clearance</td>
</tr>
<tr>
<td>CLGI</td>
<td>Insulin-independent glucose clearance</td>
</tr>
<tr>
<td>CL&lt;sub&gt;I&lt;/sub&gt;</td>
<td>Insulin clearance</td>
</tr>
<tr>
<td>df</td>
<td>Degree of freedom</td>
</tr>
<tr>
<td>DP</td>
<td>Disease progression</td>
</tr>
<tr>
<td>DPP-4</td>
<td>Dipeptidyl peptidase-4</td>
</tr>
<tr>
<td>DPP-4i</td>
<td>Dipeptidyl peptidase-4 inhibitors</td>
</tr>
<tr>
<td>DPw</td>
<td>Effect of disease progression on weight</td>
</tr>
<tr>
<td>DWGT</td>
<td>Change of weight from baseline</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50D&lt;/sub&gt;</td>
<td>Drug concentration at 50% of drug effect</td>
</tr>
<tr>
<td>EF&lt;sub&gt;D,I&lt;/sub&gt;</td>
<td>Total effect of disease progression and intervention</td>
</tr>
<tr>
<td>EFs</td>
<td>Change in weight on insulin sensitivity</td>
</tr>
<tr>
<td>EFw</td>
<td>Effect of disease progression and intervention on weight</td>
</tr>
<tr>
<td>EGP</td>
<td>Endogenous glucose production</td>
</tr>
<tr>
<td>Emax&lt;sub&gt;D&lt;/sub&gt;</td>
<td>Maximum drug effect</td>
</tr>
<tr>
<td>FDA</td>
<td>(United States) Food and Drug Administration</td>
</tr>
<tr>
<td>FDPS</td>
<td>Finnish Diabetes Prevention Study</td>
</tr>
<tr>
<td>FO</td>
<td>First-order</td>
</tr>
<tr>
<td>FOCE</td>
<td>First-order conditional estimation</td>
</tr>
<tr>
<td>FOCE INTER</td>
<td>First-order conditional estimation with interaction</td>
</tr>
</tbody>
</table>
FPG  Fasting plasma glucose
FPS  First-phase secretion of insulin
FSI  Fasting serum insulin
g  Glucose only data
GABS  Glucose absorption
GDM  Gestational diabetes mellitus
GGI  Graded glucose insulin infusion
gi  Glucose and insulin data
GLP-1  Glucagon-like peptide-1
GPR40  G-protein-coupled receptor 40
GPRG  Glucose effect on its own production
GSEN  Glucose sensitivity
Gss  Baseline glucose
GTT  Glucose tolerance test
HbA1c  Glycated haemoglobin
HDL  High density lipoprotein
HOMA  Homeostatic model assessment
IFG  Impaired fasting glucose
iIFG  Isolated impaired fasting glucose
IGI  Integrated glucose-insulin
IGT  Impaired glucose tolerance
iIGT  Isolated impaired glucose tolerance
IIV  Inter-individual variability
INCR  Incretin effect
INCR_{max}  Maximum incretin effect
INT  Intervention
INT_{RB}  Intervention effect on the rate of beta cell function
INTw  Intervention effect on weight
iOFV  Individual objective function value
IOV  Inter-occasion variability
IS  Insulin sensitivity
IS_0  Baseline insulin sensitivity, logit scale
I_{ss}  Baseline insulin concentration
ISS_0  Baseline insulin sensitivity
IVGTT  Intravenous glucose tolerance test
k_{GE1}  Rate constant of glucose effect on its own production
k_{GE2}  Rate constant of glucose effect on insulin secretion
k_{IE}  Rate constant of insulin effect on glucose clearance
k_{in}  Rate constant for production
k_{IS}  Rate constant of first phase insulin secretion
KM  Kaplan-Meier
k_{out}  Rate constant for elimination
LRT  Likelihood ratio test
MBDD  Model-based drug development
MCMP  Monte Carlo Mapped Power
MoA  Mechanism of action
MODY  Maturity-onset of diabetes in youth
MPG  Mean plasma glucose
MTT  Meal tolerance test or mean transit time
MTT-24  24 hours meal tolerance test
NLME  Nonlinear mixed effects
NGT  Normal glucose tolerance
NO  No glucose provocation
NONMEM  NON-linear Mixed Effect Modelling® software
NWPR  Normal-Inverse Wishart Prior
OFV  Objective function value
OGTT  Oral glucose tolerance test
OHA  Oral hypoglycaemic agent
PD  Probability of dropping out
PND  Probability of not dropping out
PPAR-γ  Peroxisome proliferator-activated receptor-γ
PPG  Post-prandial glucose
PROB  Probability of dropping out (logistic function)
PsN  Pearl-speak NONMEM
P_Time  Power function for time effect
Q  Inter-compartmental clearance
RB  Rate of beta cell function deterioration
REE  Relative estimation error
RESE  Residual error of early observation
RESG  Residual error of intravenous glucose
RESGPO  Residual error of oral glucose
RESI  Residual error of insulin
RSE  Relative standard error
SGLT-2  Sodium glucose co-transporter 2
SGLT-2i  Sodium glucose co-transporter 2 inhibitor
sMTT  Single meal tolerance test
SSE  Stochastic simulation and estimation
STime  Parameter related to the time effect
SU  Sulphonylurea
t_1/2  Half-life, weight component
T1DM  Type 1 diabetes mellitus
T2DM  Type 2 diabetes mellitus
TRT  Indicator variable of treatment
TZD  Thiazolidinedione
VG  Volume of distribution for glucose
V_i  Volume of distribution for insulin
V_p  Volume of distribution for peripheral glucose
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>VPC</td>
<td>Visual predictive check</td>
</tr>
<tr>
<td>WGT</td>
<td>Weight</td>
</tr>
<tr>
<td>WHIG</td>
<td>Weight-HbA1c-Insulin-Glucose</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Significance level</td>
</tr>
<tr>
<td>$\Delta$OFV</td>
<td>Difference of objective function value</td>
</tr>
<tr>
<td>$\theta_x$</td>
<td>Parameter of a linear drug effect</td>
</tr>
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</table>
Introduction

Diabetes mellitus

Diabetes mellitus is a group of metabolic disease characterized by the hyperglycaemic condition. The hyperglycaemia may be resulting from physiological defects of either insulin secretion, insulin action or both. The chronic hyperglycaemia in diabetes is associated with long-term damages, dysfunction, and failure of various organs, especially the eyes (diabetic retinopathy), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), heart, and blood vessels.\(^1\)

The origin of diabetes can be traced back to 1500 BC, through an Egyptian manuscript, which described the disease by the ‘too great emptying of urine’. In around 230 BC, Apollonius of Memphis used the word ‘diabetes’, which means ‘to pass through’, in which he and his colleagues considered diabetes as the disease of kidneys. In around the 30-50 AD, the first complete clinical description of diabetes was made appeared by Aulus Cornelius Celsus, in his important work, *De medicina*. The first person to distinguish between conditions of what known now as the diabetes mellitus and diabetes insipidus was Aretaeus of Cappadocia, a Greek physician, in the second century.\(^2,3\) In his work *On the Causes and Indications of Acute and Chronic Disease*, there were detailed descriptions and observations on diabetes mellitus, and among his writings were\(^2\):

‘Diabetes is a dreadful affliction, not very frequent among men, being a melting down of the flesh and limbs into urine. The patients never stop making water and the flow is incessant, like the opening of the aqueducts. Life is short, unpleasant and painful, thirst unquenchable, drinking excessive and disproportionate to the large quantity of urine, for yet more urine is passed…. If for a while they abstain from drinking, their mouth become parched and their body dry; the viscera seem scorched up, the patients are affected by nausea, restlessness and burning thirst, and within a short time they expire.’\(^2\)

The ancient physician and surgeon in India (400-500 AD), Sushruta and Charaka had observed that the urine from the people with diabetes attracted ants and flies, and they named the condition as ‘madhumeha’ or ‘honey urine’. They were also able to distinguish the two types of diabetes, that later to be known as type 1 (T1DM) and type 2 diabetes mellitus (T2DM). A Per-
sian physician, Avicenna (980-1037 AD), referred the diabetes condition as abnormal appetite and observed gangrene. He created a mixture of seeds (lupin, fenugreek, zedoary) as a remedy, recorded in his work, *The Canon of Medicine*. The term ‘mellitus’ was invented by a British Surgeon General, John Rollo in 1798, to differentiate the diabetes from the other form, diabetes insipidus, in which the urine was tasteless.\(^2,3\)

In the current time, globally, the prevalence of diabetes is exponentially increasing as obesity is reaching pandemic levels. In fact, the number of adults with diabetes was estimated to be 422 million in 2014 as compared to 108 million in 1980. The global prevalence in adults has almost doubled since 1980, from 4.7 to 8.5%, with the association of being overweight and obese as the strongest risk factors. In 2012, 1.5 million deaths caused by diabetes were reported, with additional deaths of 2.2 million people from cardiovascular disease, chronic kidney disease and tuberculosis, related to the higher-than-optimal blood glucose. By the latest definition, diabetes is diagnosed in three conditions: 1. fasting plasma glucose (FPG) \(\geq 7.0\, \text{mmol/L} \) (126mg/dL) or 2. 2-hour plasma glucose after 75g oral glucose tolerance test (OGTT) \(\geq 11.1\, \text{mmol/L} \) (200mg/dL) or 3. haemoglobin A1C (HbA1c) \(\geq 6.5\%\).\(^4\)

Based on the American Diabetes Association, there are other classifications of diabetes mellitus and other categories of glucose regulation, which are the T1DM, T2DM, and other specific types of diabetes, such as genetic defects of the beta-cells and insulin actions, endocrinopathies, drug or chemical-induce diabetes, infections, uncommon forms of immune-mediated diabetes, other genetics syndrome sometimes associated with diabetes and gestational diabetes mellitus. For T1DM, it is also known as insulin-dependent and juvenile-onset diabetes, which occurs in 5-10% of those who are diagnosed with diabetes. This type of diabetes occurs as a result of a cellular-mediated autoimmune destruction of the beta cell of the pancreas, and the destruction rate is quite variables among individuals. There are also some idiopathic cases of T1DM, in which some of patients have permanent insulinopenia and prone to ketoacidosis, without evidence of autoimmunity.

In T2DM, the pathophysiology ranging from predominantly insulin resistance with relatively insulin deficient to a predominant insulin secretory defect with insulin resistance. T2DM is also known as non-insulin-dependent diabetes and accounts for 90-95% of diabetes cases. Most of the patients are obese or have a high fat distribution at the abdominal area, and it is known that obesity and central obesity could cause some degree of insulin resistance. This type of diabetes is in most cases, goes undiagnosed for many years as the hyperglycaemia develops gradually and seldom becoming severe enough for the diabetes symptoms to appear. Whereas patients with T2DM may have insulin level that seems normal or elevated, a higher blood glucose levels in these patients would be expected to result in even higher insulin levels had their beta cell function been normal. This showed the de-
fect of insulin secretion in these patients, in which it is insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or anti-hyperglycaemic agents, but is seldom restored to normal. The risk of developing T2DM increases with age, obesity, and lack of physical activity.  

Besides than T1DM and T2DM, there are a few other specific types of diabetes. Genetic defects of beta-cells, for example, frequently characterized by the onset of hyperglycaemia at an early age, usually before 25 years old, which is also referred to as maturity-onset diabetes of the young (MODY). It is characterized by impaired insulin secretion with minimal or no defects in insulin action, in which abnormalities at six genetic loci on different chromosomes have been identified. Another type of diabetes, disease of the exocrine pancreas (injury to the pancreas), can be acquired through pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. Endocrinopathies can also cause diabetes, such as in the acromegaly, Cushing’s syndrome, glucagonoma and pheochromocytoma. The excessive of some hormones, for example growth hormone, cortisol, glucagon and epinephrine in those cases, can antagonize insulin secretion, which then leads to the development of diabetes. Besides, drug- or chemical-induced diabetes can also occur. Drugs such as nicotinic acid and glucocorticoids can impair insulin action, and certain toxin such as intravenous pentamidine can permanently destroy pancreatic beta cells. Diabetes caused by infections can be related to certain viruses that has been associated with the beta cell destruction, for example coxsackievirus B, cytomegalovirus, adenovirus and mumps. The uncommon form of immune-mediated diabetes exists in the form of “stiff-man” syndrome, an autoimmune disorder of the central nervous system, and anti-insulin receptor antibodies binding to the insulin receptor, and blocking the insulin binding to its receptor in target tissues. There are also other genetic syndromes that sometimes associated with diabetes, such as Down’s syndrome. In addition, there is also another type of diabetes, known as gestational diabetes mellitus (GDM), with the prevalence of 1 to 14% of pregnancies, depending in the studied population. The deterioration of glucose tolerance usually occurs in the 3rd trimester of pregnancy.

Prediabetes states

For the prediabetes or intermediate hyperglycaemia such as impaired glucose tolerance (IGT), the criteria are FPG <7.0 mmol/L (126 mg/dL) and 2–hour plasma glucose after 75g OGGT in between ≥7.8 and >11.1 mmol/L (140 mg/dL and 200 mg/dL). The other type of prediabetes state, known as the impaired fasting glucose (IFG) is when the FPG is in between 6.1 to 6.9 mmol/L (110 mg/dL to 125 mg/dL), and if measured, the 2-hour plasma glucose <7.8 mmol/L (140 mg/dL). The IGT and IFG can exist as isolated,
e.g. isolated IGT (i-IGT) and IFG (i-IFG), or in combination, e.g. combined glucose intolerance (CGI).\textsuperscript{5}

Both IGT and IFG (isolated or combined) are in the insulin-resistance states, but people with IGT have a moderate to severe muscle insulin resistance and slightly reduced hepatic insulin sensitivity, whereas people with IFG have a normal muscle insulin sensitivity but a high hepatic insulin resistant.\textsuperscript{6} Results of studies comparing the two prediabetes states are inconclusive regarding the differences between the changes in insulin resistance, first- and second-phase insulin secretions related to the beta cell function, and incretin function between IGT and IFG.\textsuperscript{5,7-15} For example, the insulin resistance was reported to be higher in IGT than IFG subjects in a study,\textsuperscript{10} but lower in some other studies.\textsuperscript{5,11,13,15} For the first-phase insulin secretion, it was reported to decrease in both IGT and IFG,\textsuperscript{11,13} but another study contradicted the result, reporting an absolute first-phase insulin secretion decrease in IFG, but not in IGT subjects.\textsuperscript{8} Similar contradictory results can be obtained for the second- or late-phase insulin secretion, in which it was reported to decrease in IGT and IFG,\textsuperscript{11} but reported to be normal in the IFG subjects in another study.\textsuperscript{13} The glucagon-like peptide-1 (GLP-1) hormone (incretin) was reported to reduce in response to oral glucose in IGT and IFG,\textsuperscript{12,14} but an increase in the GLP-1 in IFG populations was also reported.\textsuperscript{8}

The prediabetes is the earliest stage of T2DM, in which the glucose level is normal or higher than normal, but not in the range of diabetes. If left untreated, it tends to progress to T2DM and later leads to the complications in T2DM, as a result of persistent hyperglycaemia.\textsuperscript{16}

Disease progression of T2DM

As mentioned earlier, about 90-95\% of diabetes cases diagnosed in the world is the T2DM. T2DM develops as part of a wider health problem namely metabolic syndrome, which is characterized by central obesity, dyslipidaemia, hypertension and IGT.\textsuperscript{17,18} IGT is associated with hyperinsulinemia; a compensatory response for increased insulin resistance in the cells. In general, underlying insulin resistance precedes the onset of T2DM and it is also frequently found with low high density lipoprotein (HDL) as well as high triglyceride level and both conditions are the risk factors for coronary heart diseases.\textsuperscript{17,18}

Progression from healthy to overt diabetes has been explained in five stages: 1. compensation, 2. adaptation, 3. unstable early decompensation, 4. stable decompensation 5. severe decompensation.\textsuperscript{19} In stage 1 (compensation), the insulin secretion increases to compensate the increase in insulin resistance to maintain normoglycaemia, in which the beta cell mass was reported to be normal or increased. The most common examples are the insulin resistance due to obesity that accompanied by higher rate of insulin secretion and increased acute glucose-stimulated insulin secretion following
an intravenous glucose load. In stage 2, beta cells can no longer compensating as the normoglycemia can no longer be maintained. However, the glucose level continues to rise, and the changes in beta cell function and differentiation occur, especially in the form of loss of acute glucose-stimulated insulin secretion. Usually, the individuals in stage 2 usually escape the progression to diabetes for years. For the stage 3 (unstable early decompensation), the beta cells are no longer able to keep the glucose level in the prediabetes range due to a marked decline of beta cell mass and/or increase in insulin resistance. In stage 4, the beta cell mass was reported to decrease by about 50%, but the insulin secretion is still enough to prevent ketoacidosis. For the patients with T2DM, this stage lasts for a relatively long time. The last stage, which is stage 5 (severe decompensation), marked by a severe beta cells loss, and the people tend to become ketotic and truly dependent on insulin. This condition is usually occurs in patients with T1DM, but rarely occurs in T2DM.\textsuperscript{19}

In addition, there are a few clinical characterizations of diabetes disease progression, which are progression from prediabetes to overt diabetes, the lack of acute insulin response, progression to medication, loss of glycaemic control on medication, declining of beta cell function measured by homeostasis model assessment (HOMA), and progressive weight gain.\textsuperscript{20} Based on a study, progression from prediabetes to overt diabetes mostly happens in the subjects with a high baseline body mass index (BMI) and high FPG with a high increase in FPG and 2-hours glucose levels.\textsuperscript{21}

The acute insulin response has been known as the major determinant of the glucose tolerance status over time. The impairment of acute insulin response occurs when the compensatory mechanism fails to increase insulin level, leading to the failure in maintaining the normal glucose tolerance (NGT). Failure in increasing insulin secretion leads to the IGT and decreased insulin secretion leads to the development of overt diabetes.\textsuperscript{20} Additionally, the progression of diabetes could also be measured by the need for medications; and clinical trials revealed that loss of glycaemic control has happened in medication therapy. Matthews \textit{et al.} showed that patients with diabetes needed additional therapy by six years of sulphonylurea monotherapy.\textsuperscript{22} It was also revealed that individuals with higher FPG, younger, and those with lower beta cell reserve, developed higher rates of glycaemic control loss by sulphonylurea monotherapy.\textsuperscript{20,22} The declining of beta cell function is the major sign of diabetes disease progression, which begins around 12 years before diagnosis. Beta cell function decline is associated with increased hyperglycaemia despite appropriate treatment. Genetic predisposing factors, beta cell mass deficit and inflammation are the theoretical explanations of the decrease in beta cell function.\textsuperscript{20} Other than characteristics above, disease progression of diabetes is also predicted by progression of weight gain. It is known that weight loss is associated with the improvement of beta cell function and decreased the need for treatment.\textsuperscript{20,23}
Non-pharmacological intervention in T2DM

The non-pharmacological intervention has been investigated as a prevention as well as treatment for T2DM. As a prevention of T2DM, the benefit effects of lifestyle intervention such as changes of dietary intake, weight reduction management, as well as physical activities have been investigated among pre-diabetic subjects. The dietary intake examples were total fat intake <30% of energy, saturated fat intake <10% of energy, fibre intake ≥15g/1000kcal, carbohydrate intake ≥55% of total energy (%), total fat intake <30-35 energy (%), and protein intake 10-15 energy (%), and fibre intake ≥3g/MJ. The weight was aimed to be reduced in the range of 5-10% from baseline, or to be reduced if the subjects had a high BMI (≥22 or 25kg/m²). The examples of physical activity interventions were having moderate exercise ≥30 minutes/day, ≥150 minutes/week, brisk walking of 30 minutes/day, and physical activity >30-40 minutes/day. Improvements in glucose tolerance, beta cell function and insulin sensitivity were documented with the lifestyle intervention due to weight loss and increased in physical fitness. A significant reduction of the progression from IGT to T2DM was also reported in the lifestyle intervention group throughout the follow-up duration of 2 to 6 years. In a study, the cumulative 6-year incidence of T2DM was 41-46% in the intervention as compared to 68% in the control group. In other studies, the incidence of T2DM was reduced by 58% in the intervention group, after a mean of 2.8 and 3.2 years.

The treatment of T2DM today focuses on reducing the glucose concentrations measurable in blood or plasma. The reduction is done through the non-pharmacological intervention such as the diet and exercises, and commonly also drug treatments. It is known that despite interventions, independent of if it is dietary behaviour or drug therapy, the disease, once manifested progress. However, new studies have shown some remission of T2DM in some individuals after the lifestyle intervention, but the physiological mechanism is still unclear, and more works need to be done for a strong conclusion to be made. For example, in a study, the patients with T2DM in the intensive lifestyle intervention group were more likely to achieve a partial or complete remission of T2DM, with the prevalence of 11% and 7.3% at the first and the fourth year. However, it was concluded that the absolute remission rates were modest. In a separate study, 80% of the patients (8 out of 10 subjects) went into partial remission with the decrease in HbA1c by a mean of 0.5% in a 6-months program of weight loss and exercise. The authors suggested for further studies to be done on these interventions, with randomized controls and longer-term follow-up period.
Antidiabetic drug development in T2DM

Pharmacological treatment of T2DM

Most patients with T2DM will eventually require the pharmacological treatment with oral hypoglycaemic agents (OHA) and/or insulin therapy, after the non-pharmacological approach. Currently, there are a few major antidiabetic drugs classifications, which are: biguanides, sulfonylureas (SUs), thiazolidinediones (TZDs), α-glucosidase inhibitors (AGIs), meglitinides, GLP-1 receptor agonists, dipeptidyl peptidase-4 inhibitors (DPP-4i), and sodium glucose co-transporter 2 (SGLT-2) inhibitors.\textsuperscript{38,39}

**Biguanide**

Biguanide such as metformin, decreases hepatic glucose production as the main mechanism of action, and also mildly increases insulin-stimulated glucose uptake.\textsuperscript{40} Metformin was introduced in 1959 as an antihyperglycaemic agent, and the only clinically significant biguanide. It is also the most widely used antihyperglycaemic agent in the world, generally well-tolerated and typically associated with a significant reduction in HbA1C level (about 1.5%).\textsuperscript{38,39}

**Sulphonylureas (SUs)**

The main mechanism of action (MoA) of SUs is to stimulate insulin release from functioning pancreatic β-cells.\textsuperscript{40,41} Tolbutamide, the first SU was marketed in Germany in 1950. The second generation of SUs, such as glipizide and glyburide were released in 1984 in the United States, although these drugs has been in the Europe market more than 14 years earlier. In 1995, glimepiride, which is sometimes known as the third generation SU, was released in the United States. SUs are widely used, generally safe, predictable and inexpensive, although the side effect of hypoglycaemia may limit their use. A reduction of HbA1c by 1-2% can be expected with the SUs therapy.\textsuperscript{38,39}

**Thiazolidinediones (TZDs)**

The TZDs has been shown to improve insulin sensitivity through the activation of the peroxisome proliferator-activated receptor-γ (PPAR-γ) activators and increase insulin-stimulated glucose disposal in muscle.\textsuperscript{42} The first TZD agent approved by the Food Drug and Administration (FDA) was troglitazone. However, it was withdrawn in 2000 due to the side effect of liver damage. Another TZDs, which were pioglitazone and rosiglitazone were approved in 1999. In 2010, the FDA restricted the use of rosiglitazone, due to its potential to cause cardiovascular ischemia and increase the risk of bladder cancer.\textsuperscript{38,39} Later in 2013, the FDA started to ease the restriction based the result of a study, concluded that people treated with rosiglitazone
did not have an elevated risk of myocardial infarction as compared to patients taking any other anti-hyperglycaemic agents. The use of TZDs are typically associated with an increase in HbA1c level by 0.5-1.0% and is not associated with hypoglycaemia.38

α-glucosidase inhibitors
The α-glucosidase inhibitors acts as competitive, reversible inhibitors of intestinal brush border α-glucosidase, and produce the net effect of reducing absorption and production of monosaccharides in the small intestines.43 The first drug in this class was acarbose, approved by the FDA in 1995, followed by miglitol, approved in 1996. These drugs have a modest impact on HbA1c, need for multiple daily doses, and have profound gastrointestinal side effects.38,39

Meglitinides (glinides)
The meglitinides (glinides) have MoA similar to SUs, but with a different structure than the SUs. It lowers the blood glucose by stimulating the insulin release from the functioning pancreas beta cells. The effect of this drug is glucose-dependent, which diminishes at a low glucose concentration. The first agent was repaglinide, and was approved by the FDA in 1997, followed by the second one, nateglinide, approved in 2000. Glinides can cause hypoglycaemia and need for multiple daily dosing, but it is associated with the reduction of HbA1c by 1.0-1.5%.38,39

Glucagon-like peptide-1 (GLP-1) receptor agonists
The GLP-1 receptor agonist binds to GLP-1 receptor, causing increased glucose-dependent insulin secretion and glucagon suppression.40,44–46 The first GLP-1 receptor agonist that became available for clinical use is exenatide, in 2005. In 2010, the second agent, liraglutide was approved, followed by the long-acting (once weekly) exenatide, approved in 2012.38 According to the FDA press releases, a few others of GLP-1 receptor agonist were approved, which were dulaglutide (2014), albiglutide (2014) and lixisenatide (2016). The GLP-1 receptor agonists are administered subcutaneously and associated with weight loss and a 0.5-1.0% reduction in HbA1c.38

Dipeptidyl Peptidase-4 inhibitors (DPP-4i)
The DPP-4i blocks the destruction of incretin hormone, resulting in prolonged incretin activity, which in turn enhanced the glucose-dependent insulin release. Incretin regulates the glucose-dependent insulin secretion, where it increases insulin secretion when glucose level increased, particularly post-prandial.46–48 Sitagliptin (2006) was the first DPP-4i to be approved in the United States, followed by saxagliptin and linagliptin. In 2013, alogliptin was approved by the FDA. Another DPP-4i, vildagliptin was approved in
Europe, but not in the United States. These agents are taken orally, and associated with about 0.8% reduction in HbA1c.\textsuperscript{38}

**Sodium glucose co-transporter 2 (SGLT-2) inhibitors**

SGLT-2 inhibitors reduce glucose reabsorption in the kidney thus increase urinary glucose excretion.\textsuperscript{49,50} Canagliflozin was the first SGLT-2 inhibitors approved by the FDA in 2013, followed by dapagliflozin (2014)\textsuperscript{38} and empagliflozin (2016). These agents are associated with a 0.5-0.6% reduction in HbA1c, as well as a slight reduction in weight and BMI.\textsuperscript{38} Recently, based on the FDA press release in 2016, the FDA has strengthened the existing warning about the risk of acute kidney injury for canagliflozin and dapagliflozin.

**Others**

Besides than the existing major drug classification of antidiabetic agents, there are also other classes of OHAs which are currently available, but less used particularly in T2DM, including amylin agonists (pramlintide), bromocriptine and colesevelam.\textsuperscript{38,39} Another antidiabetic agent is the G-protein-coupled receptor 40 (GPR40) agonist, with the MoA of enhancing in vitro and in vivo insulin secretion as a response to glucose level (glucose-dependent effect).\textsuperscript{51,52} The agent, for example TAK-875 have reached the clinical trials, however the Phase III clinical trial of TAK-875 was recently terminated due to the side-effect of liver toxicity in patients.\textsuperscript{53,54} Despite this challenge, GPR40-based therapy provides an interesting alternative in the antidiabetics drug development. Currently, another GPR40 agents, which are the JTT-851 and P11187 are currently in the Phase II and I trials, respectively.\textsuperscript{53}

**Glucose tolerance tests in the antidiabetic drug development**

In the diabetes drug development studies with the aim to detect drug effects, various glucose provocations have been used, such as the intravenous and oral glucose tolerance test (IVGTT and OGTT, respectively), meal test, which are the single (sMTT) and 24-hour meal tolerance test (MTT-24), as well as the graded glucose insulin infusion (GGI) or the clamp test. The standard protocol involving the subjects to perform fasting for about eight to twelve hours before a baseline blood sample taken from the subjects. This is followed by the glucose provocation study of interest, with a specific glucose dose and administration route, and later, the subsequent blood sampling.\textsuperscript{55–60} Commonly, only glucose levels are measured in the pre-clinical and animal studies, and both glucose and insulin are measured in the clinical trials.
Intravenous glucose tolerance test (IVGTT)

In the IVGTT, commonly, a bolus glucose dose (0.3 g/kg) is administered over 1-2 minutes, followed by 5-minute insulin infusion 20 minutes later (for insulin-modified IVGTT). The insulin dose is added because the endogenous insulin secretion may be too low to appropriately counteract with a sudden increase of blood sugar level. Blood samples are taken pre-dose, at baseline, every 2 minutes from 2 to 55 minutes, and later at 60, 70, 80, 100, 120, 140, 160, 180, 210 and 240 minutes. In this glucose provocation test, the first 10 minutes represents the initial distribution phase of the glucose in the blood circulation. This is followed by the stimulation of insulin secretion by the pancreatic beta cells, and a peak of glucose-stimulated endogenous insulin secretion is observed, particularly in healthy subjects. The peak might be missing in patients with some degree of beta cell impairment. At this point, the endogenous glucose production by the liver ceases. An additional insulin peak can be observed, if an exogenous dose of insulin is added during the IVGTT. After that, a marked decrease of glucose concentration and increase in the glucose clearance can be observed. The IVGTT is highly reliable and reproducible, however, it is more invasive than the OGTT or the sMTT.

Oral glucose tolerance test (OGTT)

For the OGTT, there are variations in oral glucose doses and sampling times. Commonly, the standard oral glucose doses are 50, 75 and 100 g, taken orally by the subjects (usually within 5 minutes). For an individualized approach, 1.75 g/kg glucose dose is used, to a maximum of 75 g glucose. The blood samples are usually taken at baseline, and every 30 minutes later until 180, up to 240 minutes. In the clinical setting, however, only the baseline (fasting) and the 2-hour blood samples are collected, especially for diabetes screening. In the OGTT, the glucose levels increase after a lag period, reach a peak, and then declining during the elimination phase. The OGTT is widely used in the clinical setting as well as the antidiabetic drug development, as it is non-invasive, easily performed, low in cost and mimics the glucose absorption profile after a meal, with the activation of incretin effect. However, it was shown to have variability in the rate of gastric emptying and glucose absorption, and these may affect the reproducibility of the results.

Meal tolerance tests (MTTs)

The meal tolerance test includes the sMTT and MTT-24. In the sMTT, the subjects were usually given a meal equivalent to 62.5g glucose, with the blood samplings at baseline, and every 30 minutes later until 180, up to 240 minutes. In MTT-24, the glucose provocations involve three main standardized meals equivalent to 62.5g glucose per meal, and three snacks equivalent to 12.5g glucose per snack. Blood samples are drawn at baseline, every 30 minutes for 480 minutes (16 hours) and then every 120 minutes (2 hours).
until 1440 minutes (24 hours). These non-invasive MTTs are the closest to resemble the normal physiological behaviour, thus enable the researchers to study the effects of antidiabetic drugs, as close as the real-life situation. However, as the OGTT, MTTs may have a high variability in the glucose absorption and gastric emptying time.

**Graded glucose insulin infusion (GGI)**

The graded glucose insulin infusion (GGI) is also known as the euglycemic hyperinsulinemic clamp study. In this glucose provocation test, glucose solution is continuously infused at five stages each of 20 to 40 minutes, starting with 2mg/kg up to 32mg/kg with blood samples drawn at baseline, 7 minutes, and every 20 minutes from 10 to 230 minutes. The insulin level is aimed to raise until the usual postprandial level, so that the endogenous glucose production from the liver is suppressed. Therefore, it is important to add glucose infusion in various amount, to keep the glucose levels within a physiological range. This glucose provocation test is relatively invasive, labour intensive, difficult to perform and expensive. Besides, it is far from the physiological glucose and insulin regulations especially after a meal intake, as it is done in the steady-state condition. Other types of clamp studies are the hyperglycaemic and hyperinsulinemic hypoglycaemic clamp. The hyperglycaemic clamp is less commonly used than the euglycaemic clamp. The subjects are stimulated with the same level of glucose concentration throughout the study to maintain hyperglycaemia, therefore, the beta cell and peripheral insulin sensitivity, as well as the non-insulin-mediated glucose uptake can be assessed. For the hyperinsulinemic hypoglycaemic clamp, the technique is very similar to the euglycaemic hypoglycaemic clamp, except for the hypoglycaemia was maintained in this study. It is useful to address research questions related to hypoglycaemia and counter regulatory responses.

**Pharmacometrics**

Pharmacometrics has been defined as ‘the science of developing and applying mathematical and statistical methods to: (a) characterize, understand, and predict a drug’s pharmacokinetics and pharmacodynamics behaviour, (b) quantify uncertainty of information about that behaviour, and (c) rationalize data-driven decision making in the drug development process and pharmacotherapy.’ Later, a broader definition of pharmacometrics has been proposed by Barret et al. in 2008, as ‘the branch of science concerned with mathematical models of biology, pharmacology, disease, and physiology used to described and quantify interactions between xenobiotics and patients, including beneficial effects and side effects resultant from such interfaces.’
Non-linear mixed effect (NLME) models

Pharmacometrics analysis often used the non-linear mixed effects (NLME) models. It involves simultaneous estimation of the parameter’s mean and variance based on the data from all individuals.\textsuperscript{71,72} These models containing a mathematical description of a system, with a structural component describing the typical behaviour called fixed effects and a stochastic component describing the variability of the behaviour, called random effects. This combination of fixed and random effects contributes to the “mixed effects” term. For the random effects, at least three levels of variability are identified, and differentiated. One level explains the different between the parameter values for different individuals, often referred to as inter-individual variability (IIV). The second one represents the unexplained residual variability that accounts for the differences between individual prediction and observation that maybe related to measurements error, assay imprecision or model misspecification. The third level of residual error may describes the differences between occasions in the same subjects (intra-individual), often known as inter-occasion variability (IOV).\textsuperscript{73} The structural component is commonly described as compartmental models with parameters defining the components, known as population parameters.

An example of NLME model with an IIV for continuous data can be described by equation 1. In equation 2, the addition of the unexplained residual variability on a parameter for an individual is described.

\[ P_i = \theta^p * \exp(\eta_i^p) \quad \text{Eq. 1} \]

In the equation 1, \( P_i \) is the parameter value for a typical individual (i). \( \theta^p \) represents the population mean parameter value, with its log-normally distributed random effect or IIV, \( \eta_i^p \). The \( \eta_i^p \) represents the difference of the individual’s parameter from the population mean, and it is assumed to be normally distributed with a mean of zero and variance of \( \omega^2 \).

\[ Y_{ij} = f(x_{ij}, P_{ij}) + \varepsilon_{ij} \quad \text{Eq. 2} \]

Equation 2 describes the \( j^{\text{th}} \) observation in an individual, i. The \( f(x_{ij}, P_{ij}) \) is the individual prediction describes by a function determined by the parameter vector \( P_i \) (all parameters of an individual) and the independent variables \( x_{ij} \) (study design characteristics, such as time and dose). \( \varepsilon_{ij} \) represents the random effect (residual error), describing the differences between observations and individual predictions. The \( \varepsilon \) is assumed to be normally distributed with a mean of zero and variance of \( \sigma^2 \).

Besides than an additive residual error model, the proportional or both additive and proportional residual model can also be implemented. In the additive, the magnitude of residual error is the same irrespective of the pre-
dicted value (homoscedastic error). For the proportional, the magnitude of the residual error varies proportionately with the predicted values (heteroscedastic error). And for the combination of additive and residual, the error is proportional at high predictions and additive at low predictions, often referred to as the slope-intercept model.

**Maximum likelihood estimation method**

The computer software NON-linear Mixed Effect Modelling® NONMEM was used in all projects of this thesis. NONMEM uses a parametric maximum likelihood estimation method for parameter estimation, in which the parameters of a model are estimated by maximization of the extended least squared objective function. The objective function value (OFV) is approximately proportional to -2 the logarithm of the likelihood of the data. In the case of two nested models or hierarchical models, the difference in OFV is approximately $\chi^2$-distributed, with the assumption that the model is correct and the errors are normally distributed.

The likelihood ratio test (LRT) can be used to discriminate between hierarchical models. A significant improvement in model fit (e.g. with the addition of a drug effect), can be concluded when the difference of OFV ($\Delta$OFV) between models is higher to the theoretical value obtained from the $\chi^2$-distribution, with the degree of freedom (df) corresponding to the difference in the number of parameter, at a predetermined significance level ($\alpha$). For example, a decrease of 3.84 in OFV between hierarchical models with df =1 and is considered statistically significant at $\alpha = 5\%$.

**Pharmacometrics modelling in drug development**

Pharmacometrics model-based analysis is increasingly used in drug development process as a complement to traditional analysis due to its important role in accelerating the costly and time-consuming process. Several examples has shown that model-based drug development (MBDD) has reduced the sample size and the length of study with maintained study power, in clinical trials. The high study power is achieved by the fact that the model based analysis involves the simultaneous analysis of every subject’s measurements over time, with variability between individuals taken into account. On the other hand, the traditional analysis involves the comparison between a certain value (for example mean or the maximum value) of each study arm at a specific time point, thus lead to lower power.
Pharmacometrics modelling in T2DM

**Integrated models**

There are a few integrated models in diabetes that have been developed, describing the glucose-insulin regulations, as well as glucose-HbA1c relationship.

The first model describing the glucose-insulin regulation was introduced by Bergman *et al.* in 1979, called the minimal model, developed in the mongrel dogs, using the IVGTT. In the minimal model, two main parameters are described, which are the insulin sensitivity and glucose effectiveness. Insulin sensitivity was measured by the sensitivity of the glucose clearance to insulin concentration, and glucose sensitivity is the insulin-independent glucose elimination. The complexity of the glucose-insulin interaction was addressed by having a fixed input insulin concentration, while modelling the parameters related to glucose concentration. The minimal model was adapted to the nonlinear mixed-effect model by Denti *et al.* in 2010, and further developed by Largajolli *et al.* in 2012 among T2DM patients. However, this model had a limited ability for prediction and simulation purposes, as it is lacking in the simultaneous analysis of the glucose and insulin dynamics. In 2000, de Gaetano *et al.* had proposed a one-compartment model for glucose and insulin. In this model, the glucose and insulin were modelled simultaneously, with the control mechanisms between the two components. Later, in 2007, Silber *et al.* had published the integrated glucose-insulin model (IGI), developed in the healthy and T2DM patients. This model was developed with the integration of glucose and insulin as proposed by de Gaetano *et al.* with the use of tracer glucose and a higher complexity. It has also been developed in OGTT of healthy and T2DM patients, MTT (24-hours and single). The IGI model has shown to have a good mechanistic basis, with excellent simulation and estimation abilities, thus becoming a precious tool to be applied in the diabetes drug development.

The models describing the glucose and HbA1c relationship was also introduced in the form of FPG-HBA1c and mean plasma glucose (MPG)-HbA1c. Hamren *et al.* had introduced a mechanism-based pharmacodynamics model for the FPG-HbA1c, to describe the glycosylation of haemoglobin to HbA1c among patients with T2DM. Besides than FPG, the MPG relationship with HbA1c was also investigated, as in the publications by Lledo-Garcia *et al.* and Moller *et al.* in 2013. The MPG was shown to be better than FPG in predicting the HbA1c changes, as the MPG is derived from multiple glucose measurements, thus it is less sensitive to measurements errors. Besides, the changes in HbA1c was more influenced by the changes in post-prandial glucose level than the FPG. The model by Lledo-Garcia *et al.* was developed among healthy, as well as patients with T1DM and T2DM. It describes the relationship between MPG and HbA1c by using the glycosylation rate constant, with the red blood cell’s lifespan and its precur-
In the model developed by Moller et al., a good HbA1c prediction at 24-28 weeks of trial was obtained using 12-week data from the T2DM patients. This model provides a useful tool for the late-stage antidiabetic drug development, such as in improving phase III dose selection based on the phase II data.\textsuperscript{87}

**Diabetes disease progression models**

A few models have been developed to describe the progression of T2DM, which is known to progress over a long time-span. A model developed by Topp et al. has described the dynamics of glucose, insulin and beta cell mass in determining the glycaemic control among healthy subjects.\textsuperscript{88} The model was further developed by Ribbing et al. in T2DM patients, with the addition of treatment effects and impacts of disease state on insulin sensitivity and beta cell mass.\textsuperscript{89} Another model developed by Frey et al. in T2DM patients describes a linear disease progression as the changes in FPG with the drug effect.\textsuperscript{90} A more mechanistic approach was demonstrated by de Winter et al., in describing the disease progression by the integration of FPG, fasting serum insulin (FSI), HbA1c, insulin sensitivity and beta cell function in the patients with T2DM.\textsuperscript{91} This model was further developed by Choy et al., with the addition of weight component, diet and exercise effects, as well as the transit compartment for HbA1c formation with the postprandial glucose factor.\textsuperscript{92}

There is a lack of diabetes progression modelling among the prediabetes subjects, such as in the IGT population. This population is known to have different physiological characteristics than the patients with T2DM, such as in term of severity of hyperglycaemia, insulin sensitivity and resistance level as well as beta cell function. This may influence the diabetes disease progression and responses to the pharmacological and nonpharmacological interventions. In this thesis, the disease progression and nonpharmacological intervention effects were investigated in the glucose and insulin regulations among IGT subjects, using the pharmacoetics analysis.
Aims

General aim
The general aims of this thesis were to improve the design of early phase anti-diabetes drug development studies with the focus on the power to identify drug MoAs, and to characterize and quantify the progression from prediabetes to overt diabetes, both the natural progression and the progression with lifestyle intervention. These were done using pharmacometrics modelling.

Specific aims
The specific aims were:

- to compare the study power between pharmacometrics analysis using longitudinal measurements of both glucose and insulin as opposed to only glucose in: identifying a drug effect, distinguishing a correct drug MoA from the competing incorrect MoA, and identifying a secondary drug MoA in addition to a primary drug MoA.

- to investigate the most appropriate study design in phase I for the anti-diabetes drug development for several hypothetical MoAs of a study drug, using pharmacometrics model-based simulation and estimation.

- to develop the IGI model to include disease progression model for glucose and insulin in subjects with IGT and to quantify the effect of lifestyle intervention.

- to investigate natural disease progression and lifestyle intervention effects on the body weight, insulin sensitivity, and beta cell function among IGT subjects using the WHIG Model.
Methods

Data

Simulation from the prior developed model (Paper I & II)

Study design

In Paper I, data were simulated based on standardized protocol of oral glucose tolerance test (OGTT) among patients with T2DM, according to a cross-over design with placebo and study compound, using the OGTT-IGI Model. In the first occasion, a placebo was administered at time 0, followed by 75 g oral glucose 30 minutes later. A washout period was simulated after the first occasion and the second occasion started with the administration of hypothetical drug compound (50 mg) at time 0, followed by 75g oral glucose 30 minutes later. Blood samplings were simulated from baseline to every 30 minutes, until 240 minutes. Two set of datasets were produced, one with glucose and insulin, and the other with only glucose measurements.

For Paper II, same study design as in Paper I used, which was the crossover design, involving placebo and drug compound administered at time 0 for different occasion, followed by glucose provocations. The data was simulated for patients with T2DM based on six glucose provocations, which were the insulin-modified IVGTT, OGTT, sMTT, MTT-24, GGI and repeated sampling of fasting values or no oral glucose tolerance test (NO). IVGTT-IGI model\textsuperscript{56} structure was used for the simulation of IVGTT and GGI, OGTT-IGI model\textsuperscript{57} for OGTT, and MTT-IGI model\textsuperscript{64} for sMTT and MTT-24 data. In the IVGTT, a bolus glucose dose (0.3 g/kg) was administered, followed by 5-minutes insulin infusion, 20 minutes later. Blood samples were drawn at baseline, every 2 minutes from 30 to 50 minutes, every 5 minutes until 85 minutes, every 20 minutes starting from 100 to 180, 210 and 240 minutes.\textsuperscript{56} For the OGTT and sMTT, simulated patient received oral glucose dose of 75 g and 62.5 g, with the blood sampling at baseline, 30, 60, 120, 180, and 210 minutes.\textsuperscript{55,57} In the study design of MTT-24, simulated patients received three main meals containing 62.5g glucose and three snacks of 12.5g glucose. Blood sampling of both glucose and insulin was simulated at baseline, every 30 minutes for 480 minutes (16 hours) and then every 120 minutes (2hrs) until 1440 minutes (24hrs).\textsuperscript{64} In GGI, glucose solution was continuously infused at five stages each of 40 minutes, starting with 2mg/kg up to 32mg/kg with blood sampling at baseline, 7 minutes, and eve-
ry 20 minutes from 10 to 230 minutes. For NO, no glucose test simulated, but the blood samples were taken at the same time intervals as the OGTT.

**Integrated glucose-insulin (IGI) model with drug effects**

As mentioned in the previous section, the IGI model was used to simulate the data. Figure 1 was an example of the IGI-OGTT model for the simulation of OGTT data among T2DM patients with the drug effects, which was used in both Paper I and Paper II. The IGI model of IVGTT and MTT were also used in Paper II.

Five hypothetical drug MoAs were investigated on both projects: stimulatory drug effects on basal insulin secretion (BINS), insulin-independent (CLG) and insulin-dependent glucose clearance (CLGI), and inhibitory drug effects on glucose absorption (GABS) and endogenous glucose production (EGP). Two additional drug effects were studied in Paper I, which were the stimulatory drug effects on incretin activity (INCR) and glucose sensitivity (GSEN). Emax model for drug effects were used for Paper I, and linear drug effect in Paper II. Drug effects were titrated to result in 10% reduction in area under the glucose curve for study compound (AUGC) compared to placebo (AUGCPL). In Paper II, the titration was done only for sMTT, as the reference study design.

![Figure 1. Schematic presentation of integrated glucose-insulin (IGI) model in T2DM patients (Jauslin et al.) with seven hypothetical drug MoAs. Abbreviation: INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity.](image-url)
Finnish Diabetes Prevention Study (FDPS) (Paper III & IV)

For Paper III and IV, the data came from the Finnish Diabetes Prevention Study (FDPS) consisting 522 overweight (average BMI=31kg/m²), middle-aged (average age=55 years old) subjects with IGT; randomly assigned to control and lifestyle intervention groups. The subjects in the intervention group were given an intensive individual lifestyle intervention with counseling on diet, weight reduction and exercise with associated goals of intervention. The goals of interventions were 5% reduction of weight, total intake of fat and saturated fat less than 30% and 10% of energy consumed, increased in fibre intake to at least 15g per 1000kcal, and moderate exercise for at least 30 minutes per day. On the other hand, the subjects in the control group were given a general oral and written information (a two-page leaflet) about diet and exercise, at baseline and during annual visits, without specific individual program. The subjects were recruited from five different centres in Finland, namely Helsinki, Kuopio, Turku, Tampere, and Oulu.

The glucose and insulin concentrations after the OGTT was collected yearly, with sampling performed at 0, 30, 60 (for some subjects) and 120 minutes post glucose-dose. In addition, at one of the study centres, Kuopio, frequently sampled intravenous glucose tests (IVGTT) were performed for 87 of the subjects at the start (year 0) and end of the study (year 4). Glucose and insulin were sampled at 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes post-glucose dose. Subjects who developed diabetes were excluded from the study at the time of diagnosis based on the 1985 World Health Organization (WHO) technical report series after a second OGTT was performed for the diagnosis confirmation.

In Paper III, all the glucose and insulin observations from the glucose provocations (OGTT and IVGTT) performed at the Kuopio centre (101 and 82 subjects) were used, while in Paper IV, the components of baseline weight, FPG, FSI and baseline HbA1c from all centres (522 subjects) were used for the analysis.

Study power calculation (Paper I & II)

∆OFV ratio between full and reduced model (Paper I & II)

Analysis of data, both with and without insulin (Paper I) as well as for various glucose provocation study designs (Paper II) was made using the same IGI models used for the creation of the data. The analysis was conducted on both models with drug effect (full model) and without drug effect (reduced model). However, in the analysis, many parameters in the models were fixed and the IIVs were greatly reduced, especially for the system-specific parameters, as to reduce the run times. In specific, the estimated
parameters were glucose clearance (both insulin-independent, $CL_G$ and insulin-dependent, $CL_{GI}$), insulin clearance ($CL_I$), baseline glucose ($G_{SS}$), baseline insulin ($I_{SS}$), and the drug effect parameter, which were the maximum drug effect ($E_{maxD}$), drug potency ($EC_{50D}$) or linear drug effect ($\theta$) with the IIVs 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 measurements (only in Paper I), $I_{SS}$ and $CL_I$ with their associated IIVs were fixed to the published values.

Specifically, in Paper I, there are 3 main parts of investigation. For Part 1 (identify a drug effect) and Part 2 (distinguishing the correct MoA), data were simulated using one at a time of the seven hypothetical MoAs. In Part 3 (identifying a secondary MoA on top of a primary MoA), 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 the most likely combinations of MoAs were simulated.

Power was assessed as the relative difference in $\Delta OFV_{full\text{--}reduce}$ in which full represents the model with drug effect and the reduced model is without drug effect. The Likelihood Ratio Test (LRT) was used to assess the significance of adding the drug effect in the model, with the chosen $\alpha$ of 5%, and the df set to the number of differing parameters between the competing models. By using the LRT principle to discriminate between full and reduced models, the $\Delta OFV$ between full models ($OFV_{full}$) and reduced models ($OFV_{red}$) need to be at least 3.84, at $\alpha=5\%$ and df $=1$, for the drug effect to be significant. In addition, for Part 2 of Paper I, the competing models are non-hierarchical and all have the same number of parameters, so the LRT could not be used. To determine superiority between the competing models, an arbitrary critical value of 10 was used, i.e. if $\Delta OFV$ was larger than 10 in favour for the correct model, it was deemed superior, else models were deemed to be of similar quality.

In Paper I, the difference in study power between analysis with and without insulin was calculated by the relative ratio of the $\Delta OFV$ for analysis with insulin to the $\Delta OFV$ for analysis without insulin, denoted $\Delta OFV_{gi/g}$. In Paper II, study power was assessed by calculating by the relative ratio between the $\Delta OFV_{sMTT}$ for analysis of data from the sMTT design and the $\Delta OFV_{y}$ for all other designs, where $y$ denotes the designs: MTT-24, OGTT, NO, IVGTT and GGI. This ratio, denoted $\Delta OFV_{sMTT/y}$, can be interpreted as how many times more individuals are needed for an sMTT to achieve equivalent power to the compared design.

Monte Carlo Mapped Power (MCMP) Method (Paper I)

Specifically in Paper I, in addition to the $\Delta OFV_{gi/g}$, the power was also assessed using the MCMP method. This was done to compare the two different methods of calculating study power. In the MCMP method, the individual
ΔOFV (ΔiOFV) is used to discriminate between competing models. The sums of $n$ randomly sampled ΔiOFV is used to calculate ΔOFV for $n$ number of subjects. The procedure is repeated 10,000 times and the study power is assessed as the percentage of ΔOFV out of 10,000 greater than 7.81 ($\alpha=0.05$, df=3).

**Accuracy and precision of parameter estimates (Paper I & II)**

**Drug parameters (Emax$_D$ & EC$_{50D}$) (Paper I) and ($\theta_x$) (Paper II)**

In Paper I, the accuracy and precision of parameter estimation were analysed for the Emax$_D$ and EC$_{50D}$ for all drug MoAs in Part 1 of the study, and for $\theta_x$ in Paper II. The analyses were performed by using the estimated parameters values of Emax$_D$, EC$_{50D}$ or $\theta_x$, obtained from stochastic simulation and estimation (SSE) of 500 studies with 15 subjects each. The sample size of 15 subjects was chosen as it is an acceptable number of subjects in the early clinical phase of anti-hyperglycaemic trials.

In Paper I, the parameter accuracy and precision were investigated by calculating the relative estimation error (REE) of the estimated value of Emax$_D$ and EC$_{50D}$ from the full models of analysis using glucose and insulin data (gi) and the full models of analysis using only glucose data (g), as in equation 2a and 2b. It was expected that the values of Emax$_D$ and EC$_{50D}$ should be the same, with and without insulin measurements.

\[
REE (EmaxD) = \frac{EmaxD_{SSE,gi} - EmaxD_{SSE,g}}{EmaxD_{SSE,gi}} \quad \text{Eq. 2a}
\]

\[
REE (EC50D) = \frac{EC50D_{SSE,gi} - EC50D_{SSE,g}}{EC50D_{SSE,gi}} \quad \text{Eq. 2b}
\]

In Paper II, the REE of $\theta_x$ was calculate by comparing the value of $\theta_x$ obtained from the SSE with the ‘true’ value used for the simulation, as in equation 3.

\[
REE (\theta_x) = \frac{\theta_{x,SSE} - \theta_x}{\theta_x} \quad \text{Eq. 3}
\]

**Glucose area under the curve ratio (AUGC$_D$/AUGC$_{PL}$)**

Estimation of accuracy and precision of the glucose AUC were also performed, by assessing the ratio between the area under the glucose curve of drug (AUGC$_D$) and area under the glucose curve with placebo (AUGC$_{PL}$), and this ratio is denoted as AUGC$_{D/PL}$ in Paper I and AUGC$_{D/PL,Cx}$ in Paper II. The C$_x$ in AUGC$_{D/PL,Cx}$ represents different drug MoAs.
Firstly, a simulation was performed to compute the \( \text{AUGC}_D \) and \( \text{AUGC}_{PL} \) for 15 subjects, for 500 simulations of estimated placebo and full models. The simulation was done using the parameter estimates resulted from SSE, with the inclusion and exclusion of insulin data. For each simulation using SSE estimates, the mean (\( \text{AUGC}_{D/PL} \) or \( \text{AUGC}_{D/PL,Cx} \)) from 15 subjects was calculated, producing 500 mean values of \( \text{AUGC}_{D/PL} \) for \( g_i \) (\( \text{AUGC}_{gi} \)) and \( g \) (\( \text{AUGC}_{g} \)) for Paper I, and 500 mean values of \( \text{AUGC}_{D/PL,Cx} \) for Paper II.

A second simulation of placebo and full models with 15 subjects and 500 simulations was performed, to compute the \( \text{AUGC}_{D/PL} \) or \( \text{AUGC}_{D/PL,Cx} \) as the ‘true’ ratio, using the parameter values set for the original simulations. The mean \( \text{AUGC}_{D/PL} \) or \( \text{AUGC}_{D/PL,Cx} \) was calculated as in the first simulation step. REE calculated for the \( \text{AUGC}_{D/PL} \) or \( \text{AUGC}_{D/PL,Cx} \) obtained from the SSE and \( \text{AUGC}_{D/PL} \) or \( \text{AUGC}_{D/PL,Cx} \) from the true simulation, as in the equation 4a, 4b and 4c. In Paper I, the \( \text{AUGC}_{gi} \) and \( \text{AUGC}_{g} \) were expected to be the same values.

\[
\text{REE (AUGC}_{gi} = \frac{(\text{AUGC}_{D,PL}^{SSE,gi} - \text{AUGC}_{D,PL}^{True,gi})}{\text{AUGC}_{D,PL}^{True,gi}}
\]

Eq. 4a

\[
\text{REE (AUGC}_{g} = \frac{(\text{AUGC}_{D,PL}^{SSE,gi} - \text{AUGC}_{D,PL}^{True,gi})}{\text{AUGC}_{D,PL}^{True,gi}}
\]

Eq. 4b

\[
\text{REE (AUGC}_{D,PL,Cx} = \frac{(\text{AUGC}_{D,PL}^{SSE,Cx} - \text{AUGC}_{D,PL,Cx}^{True})}{\text{AUGC}_{D,PL,Cx}^{True}}
\]

Eq. 4c

Diabetes disease progression modelling

IGI model (Paper III)

The combination of IVGTT- and OGTT-IGI model among IGT subjects

The combination of intravenous and oral IGI model\(^{55-57}\) (Figure 2) was used to fit the data for baseline in describing IGI model among the IGT subjects, incorporating prior information on the parameters from the published IVGTT- and OGTT-IGI models\(^{55-57}\) using prior\(^{99}\) functionality (SPRIOR) in NONMEM 7.3\(^{74}\). The parameters with the same values for oral, intravenous, healthy volunteers and patients with T2DM were estimated with prior information.

In addition, the parameters that differed in previous populations of the IGI model between provocations or populations\(^{55-57}\) were estimated without prior information. These parameters were the \( \text{CL}_{G} \), \( \text{CL}_{GI} \), first-phase insulin secre-
tion (FPS), shape effect of glucose on its own production (GPRG), oral glucose bioavailability (BIOG), rate of first-phase insulin secretion (kIS), maximum incretin effect (INCRmax), absorbed glucose at 50% maximum incretin effect (ABSG50), as well as GSS and ISS, with their related variability. The estimation was done with a lower and upper boundary of diabetics’ and healthy subjects’ values, to mimic the physiological state of the IGT subjects that is not as optimum as healthy state, but also not as deteriorated as the patients with T2DM.16

In addition to the parameter estimates and their variability, parameter correlations were also investigated in the model, which were the off-diagonal matrix between the central glucose volume of distribution (VG), inter-compartmental glucose clearance (Q) and insulin volume of distribution (VI), as the published IGI model.55–57 Specific to IGT population in this study, an additional off-diagonal matrix was investigated for the correlation between CLGI, FPS, ISS and GSS.

All the parameter and prior values can be found in Table 5 of the results section, with the parameter uncertainty or the df related to every prior value. The $PRIOR functionality is a restricted maximum-likelihood function for constraining parameter estimation based on the prior information. The Normal-Inverse Wishart Prior (NWPRI) was used, described as a normal distribution for fixed effect parameters (typical parameters) and an Inverse-Wishart for the IIVs.99 For the priors on the fixed effect parameters, the parameter uncertainty (standard error squared (SE2)) was calculated as in the equation 5a, incorporating each typical parameter value and its relative standard error (RSE). The df was calculated using equation 5b for the priors on the IIV, taking into account the variance (ω2) of the random effect value (deviation between the individual’s and population’s value) and its RSE.

\[
SE^2 = (RSE\% / 100 \times Typical \ parameter \ value)^2 \quad \text{Eq. 5a}
\]

\[
df = 2 \times (\omega^2 / (RSE\% / 100 \times Typical \ parameter \ value)^2) + 1 \quad \text{Eq. 5b}
\]

The typical parameter values with the associated RSE were obtained from the published IGI model.55–57 The prior values, their associated variability, uncertainty and df were fixed during estimation.

**Natural disease progression and intervention effects model**

For the modelling of the natural disease progression and intervention effects, the data from baseline until the fourth year were used. The disease progression model was set to initiate 24 hours after the end of year 0 study in the dataset, to allowing the glucose and insulin concentrations to return to the baseline values before the next glucose provocations. The disease progression and intervention effects were investigated on the most reasonable parameters from the pathophysiological perspective. These parameters were
the FPS as an early insulin responsiveness in intravenous provocation, CL\textsubscript{GI} as a reflection of insulin sensitivity, \( I_{SS} \), reflecting changes in basal insulin secretion and INCR\textsubscript{max}, as the changes of incretin hormones in the diabetic state. The effect parameters were added one at a time (stepwise manner), to assess the significance of adding additional parameters in the nested models, using LRT with \( \alpha = 5\% \). Figure 2 is the illustration of the disease progression (DP) and lifestyle intervention (INT) effects in the combination of IVGTT- and OGTT-IGI.

**Figure 2.** The combination of oral and intravenous integrated glucose-insulin (IGI) model\textsuperscript{55–57} with the disease progression (DP) and intervention (INT) effects model on the first-phase insulin secretion (FPS), insulin-dependent glucose clearance (CL\textsubscript{GI}), baseline insulin concentration (\( I_{SS} \)), and maximum incretin effect (INCR\textsubscript{max}). The final model was chosen with a linear DP and INT for FPS and CL\textsubscript{GI}, and a step function from the baseline to the first year for \( I_{SS} \), in which the \( I_{SS} \) level was unchanged after the first year. No significant effects of DP and INT were found for the INCR\textsubscript{max}.

**WHIG model (Paper IV)**

In Paper IV, the IGT characteristics were described using the integrated WHIG model developed by Choy \textit{et al.}\textsuperscript{92} Some of the system parameters were fixed to the published values during the estimation process, as they were assumed to be the same in the healthy and patients’ populations. The
fixed parameters were the turn-over time of weight changes, represented in the model by the half-life of the weight compartment \((t_{1/2, \text{WGT}})\), the effect of weight change (per kg) on insulin sensitivity, represented by the scaling factor of change in weight on insulin sensitivity \((\text{Scale EFs})\), the time lifespan of HbA1c, represented by the mean transit time of HbA1c \((\text{MTT})\) and the rate of glycation in relation to FPG, i.e. the rate constant of HbA1c compartments production \((K_{in, \text{HbA1c}})\).

\[
\begin{align*}
F_{\text{DI}} &= \theta_{\text{DPW}} + \theta_{\text{INTW}} \times \text{TRT} + \eta_{\text{DPW,INTW}} \\
EF_{\text{W}} &= (100 - EF_{\text{DI}}) / 100
\end{align*}
\]

Figure 3. The investigated weight-HbA1c-insulin-glucose (WHIG) model\(^9\) with the effects of natural disease progression and lifestyle intervention \((\text{EF}_W, (\text{DPW}&\text{INTW}))\) over time on the weight component. The intervention effect on the beta cell function \((\text{EF}_B, (\text{INTB}))\) is investigated together with the natural loss of beta cell function \((B)\), that later influenced the rate of insulin production. The effect of \(\text{EF}_W\) on postprandial glucose \((\text{PPG})\) input \((\text{EF}_{\text{PPG}}, (\text{EF}_W))\) is added in the model. The fasting plasma glucose \((\text{FPG})\) and PPG drive the production of HbA1c that is described using 3 transit compartments.

The natural disease progression \((\text{DPW})\) and lifestyle intervention \((\text{INTW})\) effects over time were investigated on the weight \((\text{WGT})\) (equation 6a and 6b), beta cell function \((B)\) (equation 7a and 7b) and postprandial glucose \((\text{PPG})\) input components (equation 8a and 8b), with the expectation of decreasing weight (equation 6c), increasing beta cell function and decreasing PPG input with intervention due to lower carbohydrate intake. A decrease in PPG input is later expected to decrease the rate production of HbA1c.
\[
dWGT/dt = EF_W \times k_{inWGT} - k_{outWGT} \times WGT
\]
Eq. 6c

In equation 6a, \( \theta_{DP_w} \) and \( \theta_{INT_w} \) are the typical values of the natural disease progression and intervention effects on weight change (DP\(_W\) and INT\(_W\)). \( \eta_{DP_w,INT_w} \) is the total difference between the typical and individual value (also known as random effect) of DP\(_W\) and INT\(_W\), which is assumed to be normally distributed with the mean 0 and the variance of \( \sigma^2_{DP_w,INT_w} \). TRT is an indicator variable, taking the value of 0 for subjects in control and 1 for intervention group. The total effect of natural disease progression and intervention effects (EF\(_{DI}\)) is the total of DP\(_W\) and INT\(_W\). The effect of DP\(_W\) and INT\(_W\) on weight input (EF\(_W\)) was set to be 1 at baseline as in equation 6b, with declining body weight\(^9\) over the time as in equation 6c.

\[
RB = \theta_{RB} + \eta_{RB} + \theta_{INT,RB} \times TRT
\]
Eq. 7a

\[
B = 1/(1 + (\exp(B_0 + RB \times t/365))
\]
Eq. 7b

Equation 7a represents the rate of beta cell decrease (RB) with a typical value, \( \theta_{RB} \) and the random effect (\( \eta_{RB} \)), normally distributed with the mean 0 and the variance of \( \sigma^2_{RB} \). \( \theta_{INT,RB} \) is the lifestyle intervention effect on the rate of beta cell decrease with the typical value as \( \theta_{INTRB} \), multiplied with TRT (0 for control and 1 for intervention). The RB is added into the natural beta cell function\(^9\) equation (equation 7b), modelled as a decline from baseline beta cell function (B\(_0\)) per year on the logit scale.

\[
PPG = \theta_{PPG} \times \exp(\eta_{PPG}) + (1/EF_W) \times \text{Scale}_{PPG,EFW}
\]
Eq. 8a

In equation 8a, the postprandial glucose (PPG) is described as a typical value, \( \theta_{PPG} \), with the random effect, \( \eta_{PPG} \) (normal distribution with mean 0 and variance \( \sigma^2_{RB} \)). The effect of weight change by DP\(_W\) and INT\(_W\) (EF\(_W\)) multiplied with an estimated scale value, \( \text{Scale}_{PPG,EFW} \), and is added into the equation as the PPG lowering effect. The influence of PPG on the rate production of HbA1c is described in equation 8b.

\[
dHbA1c_{cmt1}/dt = PPG + \text{kin}_{HbA1c} \times FPG - \text{kout}_{HbA1c} \times HbA1c_{cmt1}
\]
Eq. 8b

Sensitivity and specificity analyses (Paper III)

In Paper III, sensitivity and specificity analyses were performed for the final model with DP and INT to assess the similarity between observed data and the model prediction in predicting the subjects who developed diabetes. The analyses were performed using the data from the baseline until the fourth
year. The model-based diagnosis of diabetes was made based on the diagnostic criteria of FPG or the 2-hour postprandial glucose values after a 75g OGTT. There were 3 main scenarios investigated for the model prediction, which were:

A. using all the observed data of glucose and insulin concentrations at 0, 30, 60 and 120 minutes for the OGTT and IVGTT from baseline to the fourth year
B. using the data as in the scenario A, but excluding the glucose and insulin concentrations of the IVGTT at the fourth year
C. using the data as in the scenario A, but excluding the glucose and insulin concentrations at 0, and 120-minutes for OGTT.

For the calculation of the specificity and sensitivity analyses, the subjects who developed diabetes from the model prediction were compared to the ones who developed diabetes based on the records of doctors’ diagnosis, as well as on the observed fasting and the 2-hour glucose concentrations from the data.

The sensitivity analysis represents the model ability to correctly identify the subjects who developed diabetes at the fourth year (equation 9a). For example, a value of 0.9 was translated to model’s ability to detect 90% subjects who developed diabetes (true positive) with 10% of subjects went undetected (false negative). Specificity analysis represents the model’s ability to correctly identify the subjects who did not develop diabetes until the fourth year (equation 9b). The value of 0.8, for instance, represents the ability of the model to identify 80% of the subjects who had not developed diabetes (true negative), with 20% falsely identified to develop diabetes (false positive), at the fourth year.\textsuperscript{100}

\begin{align*}
\text{Sensitivity} &= \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \\
\text{Specificity} &= \frac{\text{True negative}}{\text{True negative} + \text{False positive}}
\end{align*}

\text{Eq. 9a} \quad \text{Eq. 9b}

**Logistic regression dropout model (Paper IV)**

A logistic regression dropout model was used to describe the dropout in the data. For the subjects who dropped out, the exact date of dropout was not documented, and only the information that the dropout occurred between two subsequent visits was available. Thus, the logistic regression was chosen to develop a model for the probability of the dropout event over a time-interval, in addition to its simplicity and reliability to be implemented as a dropout model. Each potential predictor of dropout with its corresponding parameter was added one at a time, and the LRT (with \( \alpha=0.05 \)) principle was used to assess the significance of adding the predictors into the model.
Initially, only the categorical data (dropout, no dropout and right censoring) were used to develop the dropout model. A baseline risk of dropout and time changes were investigated as the most probable predictors of dropout. The time changes were described as a nonlinear model with a power function, described as the further the time since the start of baseline, the higher the probability of dropping out. Separate time slopes were investigated from the baseline until the fourth year (intervention period), and from the fourth until the sixth year (follow-up period), in view of the probability of dropping out might be higher during the follow-up period than during the active intervention phase.

In addition to the baseline risk and time factors, the observed body weight and waist circumference with their changes from baseline, groups (control or intervention), age, as well as gender were also investigated as the predictors of dropout. The model described the probability of dropout event to occur from one visit to the other (every 365th day), which was restricted to be 0 and 1, using the logit transformation. Besides, in relation to the design of the study, the dropout model was also included the estimation of the probability of dropout event to occur when the FPG $\geq 7.8$ mmol/L, and a zero probability of dropout at baseline (before the first year).

After the dropout model were established using only the categorical data, a simultaneous model fitting with the continuous and categorical data was performed. The predictors and covariates values from the model prediction were used in the simultaneous dropout modelling, with the addition of the predicted insulin sensitivity value. The final logistic regression dropout model for the simultaneous analysis was represented by the Equation 10a to 13c in the results section.

Data analyses and model evaluation

Simulation of data and data analysis was done using nonlinear mixed-effects modelling with first-order conditional estimation method (FOCE) (Paper I and II) and FOCE with interaction (FOCE INTER) (Paper III) and LAPLACE (Paper IV) in NONMEM version 7.3 with ADVAN13 as the differential equation solver. Dataset for NONMEM and all graphs were prepared and produced using R software with the ggplot2 package. A bootstrap run with 100 samples (Paper III) and 500 samples (Paper IV) was used to assess the uncertainty in the parameter estimates. In Paper I and II, the SSE and MCMP as implemented in PsN was used to generate 500 samples of reduced and full models, and later used in the investigations of the accuracy and precision of the parameter estimation. All the calculations for the parameter accuracy and precision were performed using R software.

The visual predictive checks (VPCs) for the continuous data in different glucose provocations, subject groups and years (Paper III and IV) were
performed as implemented in PsN (version 4.6.12) with 500 simulations. In Paper III, the censoring option\textsuperscript{105} for the subjects who develop diabetes based on the FPG or the 2-hour plasma glucose concentrations from the model prediction was added in the VPC. This was done in order to mimic the original study design, in which the subjects who developed diabetes were excluded at the time of diagnosis\textsuperscript{24}. Additionally, in Paper IV, a VPC with 100 simulations was performed for analysis of the categorical data only. The visualization of the data and results was done using Xpose4 (version 4.5.0).\textsuperscript{106–108} The LRT was used to discriminate between nested models, with the chosen $\alpha=5\%$. Based on LRT, the OFV is assumed to be chi-square distributed, and a decrease of 3.84 in OFV between hierarchical models with df $=1$ is considered statistically significant at $\alpha=5\%$. The final model was chosen based on the lowest OFV, the best VPCs, specificity and sensitivity analyses (Paper III) as well as the scientific plausibility.
Results

Study power

Inclusion vs. exclusion of insulin measurements (Paper I)

Part 1: Identify a drug effect

The study power to identify a drug effect, with and without insulin measurements is shown in Table 1. The power to detect a drug effect was higher when insulin measurements were included in the analysis in all drug effect. The largest difference in the study power was for detecting the drug effect on stimulating glucose sensitivity, $\Delta OFV_{gi/g} = 2.1$, i.e. 2.1 times more individuals are needed to achieve the same power if insulin is excluded from the analysis. This is followed by incretin effect and basal insulin secretion ($\Delta OFV_{gi/g} = 1.5$), glucose absorption ($\Delta OFV_{gi/g} = 1.3$), and glucose clearance and EGP ($\Delta OFV_{gi/g} = 1.2$).

The comparison of $\Delta OFV_{gi/g}$ and MCMP method in assessing the study power is summarized in the Table 1. The number of subjects needed at 95% study power at $\alpha = 5.0\%$, 1.0% and 0.1%, with df=3 for the 7 drug MoAs from the MCMP method was recorded as comparisons. The ratio of number of subjects needed to detect a drug effect with insulin relative to without insulin was similar to those obtained using $\Delta OFV_{gi/g}$. For a drug effect on increasing glucose sensitivity, the study power was extremely high; 99% study power was achieved with only 3 subjects at $\alpha=0.1\%$ when including insulin in the analysis, thus the granularity to assess the ratio of individuals was too small. In general, a high study power (95%) was achieved when using pharmacometrics model-based analysis.
Table 1. (Part 1) The relative study power to detect a drug effect, presented as ratio of needed individuals for the same power with and without insulin. The table lists the ratio for the analysis with insulin relative to without insulin, ΔOFVgi/g as well as the number of subjects needed to achieve a power of 95% at 5%, 1%, 0.1% significance level (α), for all investigated drug MoAs.

<table>
<thead>
<tr>
<th>Assessed with ΔOFVgi/g</th>
<th>INCR</th>
<th>BINS</th>
<th>CLG</th>
<th>CLGI</th>
<th>EGP</th>
<th>GABS</th>
<th>GSEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of subjects</td>
<td>1.5</td>
<td>1.5</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.3</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessed with MCMP at 95% power</th>
<th>INCR</th>
<th>BINS</th>
<th>CLG</th>
<th>CLGI</th>
<th>EGP</th>
<th>GABS</th>
<th>GSEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects, α=5% with insulin</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Ratio of subjects (with/without insulin), α=5%</td>
<td>1.3</td>
<td>1.5</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>&gt; 1.3</td>
</tr>
<tr>
<td>No of subjects, α=5% without insulin</td>
<td>5</td>
<td>6</td>
<td>13</td>
<td>7</td>
<td>14</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>No of subjects, α=1% with insulin</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>7</td>
<td>12</td>
<td>5</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Ratio of subjects (with/without insulin), α=1%</td>
<td>1.5</td>
<td>1.4</td>
<td>1.4</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>&gt; 1.7</td>
</tr>
<tr>
<td>No of subjects, α=1% without insulin</td>
<td>6</td>
<td>7</td>
<td>15</td>
<td>8</td>
<td>14</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>No of subjects, α=0.1% with insulin</td>
<td>5</td>
<td>6</td>
<td>13</td>
<td>9</td>
<td>15</td>
<td>7</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Ratio of subjects (with/without insulin), α=0.1%</td>
<td>1.5</td>
<td>1.3</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td>&gt; 2.0</td>
</tr>
<tr>
<td>No of subjects, α=0.1% without insulin</td>
<td>7</td>
<td>8</td>
<td>17</td>
<td>10</td>
<td>17</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

Part 2: Distinguishing the correct MoAs
As summarized in Table 2, the power to identify the correct drug MoAs versus incorrect was largely affected by the exclusion of insulin for most cases. The most pronounced difference in power was observed for drug MoAs on insulin-dependent glucose clearance versus increasing incretin activity and basal insulin secretion. In these cases, more than 100 subjects were needed when excluding insulin, indicating perhaps a non-identifiability of drug effect without insulin measurements. In other cases, more than four times sample size was needed when analysing without insulin than analysing with insulin, to achieve an equal power in distinguishing drug MoAs of basal insulin secretion from insulin-dependent glucose clearance, incretin effect from basal insulin secretion and insulin-dependent glucose clearance, insu-
lin-independent glucose clearance from basal insulin secretion, as well as glucose sensitivity from incretin effect and basal insulin secretion. In addition, at least 1.5 times fewer subjects were needed in other cases, to distinguish true from false MoAs with the inclusion of insulin measurements.

The study power however, may not be affected by insulin measurements to distinguish drug MoAs of insulin-dependent from insulin-independent glucose clearance, EGP and glucose absorption, and EGP from glucose absorption. Besides, the correct drug MoA of increasing insulin-independent glucose clearance could not be discriminate from the incorrect of decreasing EGP, even when insulin measurements were included in the study. This conclusion was based on more than 100 subjects were needed, both when including and excluding insulin measurements.

Table 2. (Part 2) The relative study power to distinguish correct drug mechanism of action (MoA) from incorrect, represented as ratio of subjects needed to achieve a same power with and without insulin. The table lists the ratio of ΔOFV for the analysis with insulin relative to without insulin, ΔOFVgi/g.

<table>
<thead>
<tr>
<th>Correct MoA</th>
<th>INCR</th>
<th>BINS</th>
<th>CLG</th>
<th>CLGI</th>
<th>EGP</th>
<th>GABS</th>
<th>GSEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCR</td>
<td>-</td>
<td>4.7</td>
<td>1.7</td>
<td>4.1</td>
<td>2.5</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>BINS</td>
<td>*</td>
<td>-</td>
<td>1.8</td>
<td>6.0</td>
<td>2.5</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>CLG</td>
<td>3.8</td>
<td>4.7</td>
<td>-</td>
<td>1.5</td>
<td>**</td>
<td>1.6</td>
<td>2.4</td>
</tr>
<tr>
<td>CLGI</td>
<td>*</td>
<td>*</td>
<td>1.0</td>
<td>-</td>
<td>1.0</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>EGP</td>
<td>2.9</td>
<td>3.1</td>
<td>1.0</td>
<td>1.4</td>
<td>-</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>GABS</td>
<td>3.7</td>
<td>3.8</td>
<td>1.2</td>
<td>1.4</td>
<td>1.2</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>GSEN</td>
<td>4.4</td>
<td>4.5</td>
<td>2.5</td>
<td>3.5</td>
<td>2.6</td>
<td>3.1</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviation: MoA = Mechanism of action, INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity.

* >100 subjects were needed to distinguish the drug effect without insulin
** >100 subjects were needed to distinguish the drug effect with and without insulin

Part 3: Identifying a secondary drug MoA on top of a primary MoA
As shown in Table 3, the overall power to detect a secondary true drug MoA was higher with the use of glucose and insulin as opposed to only glucose measurements in the analysis. This is most pronounced for detecting a secondary drug MoA of basal insulin secretion on top of the primary drug MoA of incretin effect, and insulin-dependent glucose clearance on top of basal insulin secretion. More than 100 subjects were needed to detect the secondary drug MoAs from the primary ones when excluding insulin in these cases, indicating perhaps a non-identifiability of the secondary effect without insulin. Furthermore, a major difference, at least twice as many subjects were needed when insulin measurements were excluded from the study, to detect
insulin-dependent glucose clearance on top of glucose sensitivity ($\Delta\text{OFV}_{gi/g} = 3.7$), EGP on top of basal insulin secretion ($\Delta\text{OFV}_{gi/g} = 2.1$), glucose absorption on top of incretin effect ($\Delta\text{OFV}_{gi/g} = 8.1$) and glucose sensitivity on top of all tested primary drug MoAs (range $3.0 < \Delta\text{OFV}_{gi/g} < 6.0$). In other cases, a slight increase of power to detect secondary drug MoA was observed, which were for insulin-independent on top of insulin-dependent glucose clearance ($\Delta\text{OFV}_{gi/g} = 1.4$), EGP on top of incretin effect and glucose sensitivity ($\Delta\text{OFV}_{gi/g} = 1.6$ and $1.8$, respectively), as well as glucose absorption on top of insulin-independent glucose clearance ($\Delta\text{OFV}_{gi/g} = 1.5$).

There was no difference of study power to detect insulin-independent glucose clearance on top of EGP, and EGP on top of glucose clearance. In addition, in identifying a secondary drug MoA on insulin-independent glucose clearance on top of primary MoA on EGP, more than 100 subjects were required, independent of sampling insulin.

Table 3. (Part 3) The relative study power to detect correct secondary correct drug effect with the primary already included, represented as ratio of subjects needed to achieve a same power without and with insulin. The table lists the ratio of $\Delta\text{OFV}$ for the analysis with insulin relative to without insulin, $\Delta\text{OFV}_{gi/g}$.

<table>
<thead>
<tr>
<th>Secondary MoA</th>
<th>Competing Incorrect MoA</th>
<th>$\Delta\text{OFV}_{gi/g}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BINS</td>
<td>*</td>
<td>BINS</td>
</tr>
<tr>
<td>CLG</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>CLGI</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>EGP</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>GABS</td>
<td>8.1</td>
<td>6.0</td>
</tr>
<tr>
<td>GSEN</td>
<td>4.3</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Abbreviation: MoA = Mechanism of action, INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity.

* >100 subjects were needed to detect the secondary drug MoA without insulin

** >100 subjects were needed to detect the secondary drug MoA with and without insulin

Most appropriate study design based on the drug MoAs (Paper II)

Table 4 shows the study power of each study design as a relative ratio to the study power of sMTT model. As mentioned earlier, this ratio is the representation of how many times more individuals are needed for a sMTT to achieve equivalent power to the compared designs. Intravenous provocations were always more powerful than oral provocations except for MTT-24 in certain MoAs. The power of MTT-24 was higher than sMTT for all MoAs except for increasing insulin-independent glucose clearance. Repeated fast-
ing samples or NO had for many MoAs higher power than sMTT. Notable though is that safety was not assessed in this study, and no glucose provocation may be associated with more hypoglycaemia than other designs. The MTT-24 and OGTT were the least powerful study designs for drug effect on increasing insulin-independent glucose clearance, potentially due to the stimulation of insulin release at a higher rate by incretin activity made the insulin–independent glucose clearance less important.

Table 4. The study power of each model for each drug effect is described as a ratio relative to the study power of single meal tolerance test (sMTT).

<table>
<thead>
<tr>
<th>Drug effects</th>
<th>Study designs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sMTT</td>
</tr>
<tr>
<td>BINS</td>
<td>1</td>
</tr>
<tr>
<td>CLG</td>
<td>1</td>
</tr>
<tr>
<td>CLGI</td>
<td>1</td>
</tr>
<tr>
<td>EGP</td>
<td>1</td>
</tr>
<tr>
<td>GABS</td>
<td>1</td>
</tr>
</tbody>
</table>

**Abbreviation:** IVGTT = intravenous glucose tolerance test; GGI = graded glucose infusion; NO = no provocations test; OGTT = oral glucose tolerance test; sMTT = single meal tolerance test; MTT-24 = 24-hours meal tolerance test, INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity. Yellow represents the most powerful, and the green represents the second most powerful designs.

Accuracy and precision of parameter estimates (Paper I & II)

Drug parameter $E_{\text{maxD}}$ & $E_{50D}$ and $\theta_x$

In Paper I, an acceptable accuracy and precision of the parameter $E_{\text{maxD}}$ without insulin measurements was observed for the drug effects with MoA related to glucose: glucose clearance, EGP and glucose absorption. For the $E_{50D}$, the only drug MoA where accuracy was acceptable without insulin measurements was for glucose absorption. The precision of $E_{50D}$ for this drug MoA was however poor. Moreover, in all other drug MoAs for both parameters, the estimates were both biased and poor in precision. The results are illustrated in Figure 4.

For Paper II, the precision and accuracy of the $\theta_x$ parameter are visualized in Figure 5. The parameter estimation derived from OGTT, IVGTT and GGI were overall good for all MoAs. Thus, the most powerful study designs, albeit including the two most invasive, were also producing accurate and precise estimates. The precision and accuracy of MTT-24 and sMTT were dependent on the MoA of the drug. The precision of MTT-24 was the best
for all MoAs. The worst precision and accuracy in all MoAs were the sMTT for drug effect on insulin-dependent glucose clearance, and NO for drug effect on insulin-independent glucose clearance, despite these designs being rather powerful.

**Figure 4.** (Part 1) Relative estimation error (REE) of the drug parameters of maximum drug effect (EMAXD) and drug concentration at 50% EMAX (EC50D). Abbreviation: SSE = stochastic simulation and estimation, INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity.
Figure 5. A descriptive representation of the distribution of relative estimation error of each study design model for five investigated drug MoAs. The different study design models are intravenous glucose tolerance test (IVGTT), oral glucose tolerance test (OGTT), single meal tolerance test (sMTT), 24-hours meal tolerance test (MTT-24), graded glucose infusion (GGI) and no-provocations test (NO). **Abbreviation:** **BINS** = basal insulin secretion, **CLG** = insulin-independent glucose clearance, **CLGI** = insulin-dependent glucose clearance, **EGP** = glucose production, **GABS** = glucose absorption. Yellow represents the most powerful, and green represents the second most powerful designs.
Glucose area under the curve ratio ($\text{AUGC}_{\text{D/PL}}$)

The $\text{AUGC}_{\text{D/PL}}$ for Paper I was investigated and reported in Figure 6. The $\text{AUGC}_{\text{D/PL}}$ was found to be estimated with an adequate accuracy to the true simulation value and with a good precision for most drug effects, both including and excluding insulin data in the analysis, despite the inaccuracy and imprecision of the $\text{EmaxD}$ and $\text{EC}_{50D}$.

In addition, the same observation found in Paper II, in which despite the differences between the estimation and simulation of $\theta_x$ values, both models can precisely simulate the same $\text{AUGC}_{\text{D/PL,Cx}}$ as shown in Figure 7. The worst accuracy was NO for drug effects on insulin-dependent glucose clearance. Although $\theta_x$ estimated in sMTT were the least precise and accurate for drug MoAs on insulin-dependent glucose clearance, sMTT can still simulate $\text{AUGC}_{\text{D/PL,Cx}}$ precisely as simulation models. The same observation was seen for NO when the drug effect is on insulin-independent glucose clearance. NO can simulate plasma glucose $\text{AUGC}_{\text{D/PL,Cx}}$ with good precision and accuracy, but it was still the worst among all other models for this drug MoA. In conclusion, the study design with the highest power for a drug MoA was not always the most precise neither was the most accurate one. The most powerful, accurate and precise study designs are the intravenous ones which are also the most invasive. From power perspective, MTT-24 is a good alternative, unless we are expecting effect on insulin-independent glucose clearance like SGLT-2-inhibitors, but it seems to be resulting in a biased parameter estimate.
Figure 6. (Part 1) Relative estimation error (REE) for AUGC ratio, in seven hypothetical drug MoAs. Abbreviation: Glu + Ins = glucose and insulin data, Glu = glucose only data, INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity.
Figure 7. A descriptive representation of the distribution of relative estimation error of AUC ratio for five investigated drug MoAs. The different study design models are intravenous glucose tolerance test (IVGTT), oral glucose tolerance test (OGTT), single meal tolerance test (sMTT), 24-hours meal tolerance test (MTT-24), graded glucose infusion (GGI) and no-provocations test (NO). Abbreviation: BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-independent glucose clearance, EGP = glucose production, GABS = glucose absorption. Yellow represents the most powerful, and green represents the second most powerful designs.
Diabetes disease progression modelling

IGI model (Paper III)

The combination of IVGTT- and OGTT-IGI model among IGT subjects

Final parameter estimates for the subjects with IGT were summarized in Table 5, under the baseline data final estimates. In general, the fixed effect parameters with prior information were similar with or only slightly deviated from the initial values. For the parameters without prior information, the values were estimated to be in between the values of healthy subjects and patients with T2DM, except for the rate constant of glucose effect on its own production ($k_{GE1}$) and $CL_G$. The $k_{GE1}$ was fixed to the previously published value of 0.0573 min$^{-1}$ as it was estimated to be unreasonably higher than the published one. $CL_G$ was fixed to the published diabetic value due its tendency to reach the lower boundary during estimation. The $CL_{GI}$, FPS, and GPRG were closer to diabetic values, whereas $BIO_G$ was closer to healthy subjects’. The values for $I_{SS}$ and $G_{SS}$ were in agreement with the definition of IGT population. An adequate model description of the data on the parameters level was achieved, indicated by the reasonable parameter estimations in the IGI model.

For the inter-individual variability, similar parameter values with the ones published were estimated in most cases. For some parameters as $V_G$, IN-$CR_{max}$, $CL_{I}$, $V_P$, effect delay rate constant of glucose on insulin secretion ($k_{GE2}$), shape effect of glucose on insulin secretion (IPRG) and effect delay rate constant of insulin effect on glucose clearance ($k_{IE}$) were decreased from the published values. Parameter correlation between the $Q$ with $V_G$ and $V_I$ was increased in the IGT subjects, whereas the correlation between $V_G$ and $V_I$ was lower.

Natural disease progression and intervention effects model

Natural disease progression effect

The effects of natural disease progression and intervention on chosen parameters are summarized in Table 5, as all final parameter estimates. The FPS and $CL_{GI}$ were decreased by 3.0% and 8.1% per year in all subjects as a result of the natural disease progression. The $I_{SS}$ was observed to increase by 68% from baseline to Year 1, and remain unchanged after the first year.

Lifestyle intervention effect

On top of the natural disease progression, the lifestyle intervention increased the FPS value by 2.9% per year, producing a net effect of 0.1% decrease per year. This can be interpreted as a much slower deterioration of the acute response of beta cell function to produce insulin following an IVGTT. The $CL_{GI}$ had an increment of 6.0% per year with the lifestyle intervention, producing a 2.1% decrease per year, representing a slower deterioration of insu-
lin sensitivity with intervention. The $I_{SS}$ was affected by the intervention with a net effect of 153% increase from baseline to the first year and remained constant after that, reflecting the improvement of beta cell function to secrete insulin during the specific period.

No significant effects of natural disease progression and lifestyle intervention were found for the $\text{INCR}_{\text{max}}$. The model fit was no better than the chosen final model, in which an insignificant OFV improvement and worse VPCs were observed. Besides, the specificity and sensitivity results were also worse than the chosen final model, indicating a poor model prediction.

Table 5. Final parameter estimates for only baseline and all data (baseline until the fourth year) in the combination of integrated glucose-insulin for intravenous and oral glucose tolerance test (IVGTT-OGTT-IGI) among the subjects with impaired glucose tolerance (IGT) with the use of prior information from the published IGI model.\textsuperscript{55–57}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose</th>
<th>Initial estimates/ *Prior</th>
<th>Uncertainty / df</th>
<th>Final estimates (RSE, %)\textsuperscript{I}</th>
<th>Final estimates (RSE, %)\textsuperscript{I}</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_G, L$</td>
<td>Central volume of distribution</td>
<td>9.33* 0.313376</td>
<td>8.92 (2.3)</td>
<td>9.13 (1.4)</td>
<td></td>
</tr>
<tr>
<td>$V_p, L$</td>
<td>Peripheral volume of distribution</td>
<td>8.56* 0.183184</td>
<td>8.01 (3.0)</td>
<td>8.78 (0.6)</td>
<td></td>
</tr>
<tr>
<td>$\text{CL}_{GI}, L/min$</td>
<td>Insulin-independent clearance</td>
<td>0.0287\textsuperscript{†}</td>
<td>-</td>
<td>0.0287\textsuperscript{†}</td>
<td></td>
</tr>
<tr>
<td>$\text{CL}_{GI}, L/min/ (mu/L)$</td>
<td>Insulin-dependent clearance</td>
<td>(0.00297, 0.00829)\textsuperscript{†}</td>
<td>-</td>
<td>0.00405 0.00389</td>
<td></td>
</tr>
<tr>
<td>$Q, L/min$</td>
<td>Inter-compartmental clearance</td>
<td>0.442* 0.005001</td>
<td>0.35 (10.1)</td>
<td>0.25 (9.6)</td>
<td></td>
</tr>
<tr>
<td>$k_{GE1}, min^{-1}$</td>
<td>Effect delay rate constant, glu. on its own prod.</td>
<td>0.0573\textsuperscript{†} 0.000040</td>
<td>0.0573\textsuperscript{†}</td>
<td>0.0573\textsuperscript{†}</td>
<td></td>
</tr>
<tr>
<td>$k_{GE2}, min^{-1}$</td>
<td>Effect delay rate constant, glu. on ins. Sec.</td>
<td>0.0289* 0.000016</td>
<td>0.0335 (3.6)</td>
<td>0.0307 (3.2)</td>
<td></td>
</tr>
<tr>
<td>GPRG (-)</td>
<td>Shape effect, glu on its own production</td>
<td>(-2.79,0)\textsuperscript{†}</td>
<td>-</td>
<td>-0.587 (38.2) -1.37 (11.7)</td>
<td></td>
</tr>
<tr>
<td>IPRG (-)</td>
<td>Shape of effect, glu. on ins. secretion</td>
<td>1.42* 0.029036</td>
<td>1.59 (2.4)</td>
<td>1.86 (1.5)</td>
<td></td>
</tr>
<tr>
<td>$\text{BIOG}, %$</td>
<td>Bioavailability</td>
<td>(0.722, 0.811)\textsuperscript{†}</td>
<td>-</td>
<td>0.754 (6.2) 0.723 (3.0)</td>
<td></td>
</tr>
<tr>
<td>MTT, min</td>
<td>Mean transit time of glucose absorption</td>
<td>34.9* 14.738</td>
<td>44.2 (4.1)</td>
<td>37.9 (2.9)</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>Number of glucose transit compartment</td>
<td>1.27* 0.0929</td>
<td>1.8 (8.9)</td>
<td>0.9 (9.0)</td>
<td></td>
</tr>
<tr>
<td>$\text{INCR}_{\text{max}}$</td>
<td>Maximal incretin effect</td>
<td>1.47\textsuperscript{†}</td>
<td>-</td>
<td>1.93 (19.7) 1.26 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Value</td>
<td>Value (SD)</td>
<td>Value (SD)</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline glucose</strong></td>
<td>G&lt;sub&gt;ss&lt;/sub&gt;, mg/dL</td>
<td>105.6&lt;sup&gt;§&lt;/sup&gt;</td>
<td>108 (1.1)</td>
<td>107 (1.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td>V&lt;sub&gt;t&lt;/sub&gt;, L</td>
<td>6.09&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.300414</td>
<td>4.0 (2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;L&lt;/sub&gt;, L/min</td>
<td>1.22&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.003721</td>
<td>0.805 (1.7)</td>
<td>0.852 (2.1)</td>
</tr>
<tr>
<td></td>
<td>k&lt;sub&gt;IL&lt;/sub&gt;, min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.0213&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.0000008</td>
<td>0.0218 (5.6)</td>
<td>0.0155 (9.0)</td>
</tr>
<tr>
<td></td>
<td>k&lt;sub&gt;ts&lt;/sub&gt;, min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.384&lt;sup&gt;§&lt;/sup&gt;</td>
<td>-</td>
<td>0.824 (12.8)</td>
<td>0.678 (8.1)</td>
</tr>
<tr>
<td></td>
<td>FPS, mU</td>
<td>(0.704)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>-</td>
<td>119 (12.9)</td>
<td>155 (6.1)</td>
</tr>
<tr>
<td></td>
<td>I&lt;sub&gt;ss&lt;/sub&gt;, mU/L</td>
<td>13.5&lt;sup&gt;§&lt;/sup&gt;</td>
<td>-</td>
<td>13.5 (4.7)</td>
<td>6.35 (2.8)</td>
</tr>
<tr>
<td></td>
<td>I&lt;sub&gt;ss&lt;/sub&gt;,NT, mU/L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.11 (3.5)</td>
</tr>
<tr>
<td><strong>Disease progression and intervention effects</strong></td>
<td>DP_FPS Disease progression on FPS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.030 (35.3)</td>
</tr>
<tr>
<td></td>
<td>DP_CL&lt;sub&gt;Gi&lt;/sub&gt; Disease progression on CL&lt;sub&gt;Gi&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.081 (40.3)</td>
</tr>
<tr>
<td></td>
<td>DP_INCR&lt;sub&gt;max&lt;/sub&gt;Disease progression on INCR&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DP_I&lt;sub&gt;ss&lt;/sub&gt; Disease progression on I&lt;sub&gt;ss&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.683 (14.9)</td>
</tr>
<tr>
<td></td>
<td>INT_FPS Intervention effect on FPS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.029 (79.0)</td>
</tr>
<tr>
<td></td>
<td>INT_CL&lt;sub&gt;Gi&lt;/sub&gt; Intervention effect on CL&lt;sub&gt;Gi&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.060 (68.4)</td>
</tr>
<tr>
<td></td>
<td>INT_INCR&lt;sub&gt;max&lt;/sub&gt; Intervention effect on INCR&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>INT_I&lt;sub&gt;ss&lt;/sub&gt; Intervention effect on I&lt;sub&gt;ss&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.849 (24.4)</td>
</tr>
<tr>
<td><strong>Residual error</strong></td>
<td>RESG, % Residual error, iv glucose</td>
<td>7.32&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000003</td>
<td>3.81 (4.1)</td>
<td>6.5 (3.4)</td>
</tr>
<tr>
<td></td>
<td>RESGPO, % Residual error, oral glucose</td>
<td>8.58&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000027</td>
<td>9.15 (2.0)</td>
<td>14.8 (1.4)</td>
</tr>
<tr>
<td></td>
<td>RESI, % Residual error, insulin</td>
<td>25.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000229</td>
<td>14.9 (3.8)</td>
<td>25.2 (3.0)</td>
</tr>
<tr>
<td></td>
<td>RESE (-) Residual error, early</td>
<td>3.31&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.483164</td>
<td>1.64 (5.9)</td>
<td>1.25 (3.7)</td>
</tr>
<tr>
<td><strong>Inter-individual variability, %</strong></td>
<td>Glucose V&lt;sub&gt;G&lt;/sub&gt; Central volume of distribution</td>
<td>30&lt;sup&gt;*&lt;/sup&gt;</td>
<td>63</td>
<td>24.4 (3.8)</td>
<td>23.8 (5.4)</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;p&lt;/sub&gt; Peripheral volume of distribution</td>
<td>30&lt;sup&gt;*&lt;/sup&gt;</td>
<td>27</td>
<td>28.2 (16.4)</td>
<td>97.0 (37.3)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Unit</td>
<td>Prior</td>
<td>Fixed</td>
<td>Estimated</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>IOV, $V_p$</td>
<td>Peripheral volume of distribution, IOV</td>
<td></td>
<td>15*</td>
<td>50.6 (21.6)</td>
<td>93.7 (18.8)</td>
</tr>
<tr>
<td>$CL_G$</td>
<td>Insulin-independent clearance</td>
<td></td>
<td>59†</td>
<td>59†</td>
<td>59†</td>
</tr>
<tr>
<td>$CL_{GI}$</td>
<td>Insulin-dependent clearance</td>
<td></td>
<td>46</td>
<td>46.5 (10.1)</td>
<td>65.0 (26.1)</td>
</tr>
<tr>
<td>$Q$</td>
<td>Inter-compartmental clearance</td>
<td></td>
<td>85*</td>
<td>83.9 (8.8)</td>
<td>77.6 (8.1)</td>
</tr>
<tr>
<td>$k_{GE2}$</td>
<td>Effect delay rate constant, glu. on ins.</td>
<td></td>
<td>85*</td>
<td>58.1 (9.6)</td>
<td>59.9 (13.2)</td>
</tr>
<tr>
<td>IPRG</td>
<td>Shape of effect, glu. on ins. secretion</td>
<td></td>
<td>35*</td>
<td>28.1 (14.0)</td>
<td>20.6 (9.5)</td>
</tr>
<tr>
<td>MTT</td>
<td>Mean transit time of glucose absorption</td>
<td></td>
<td>11*</td>
<td>19.3 (26.3)</td>
<td>28.1 (32.5)</td>
</tr>
<tr>
<td>$INCR_{max}$</td>
<td>Maximal incretin effect</td>
<td></td>
<td>55§</td>
<td>19.7 (88.5)</td>
<td>49.4 (19.3)</td>
</tr>
<tr>
<td>$ABSG_{50}$</td>
<td>Absorbed glucose at 50% $INCR_{max}$</td>
<td></td>
<td>114§</td>
<td>131.9 (121.4)</td>
<td>137.5 (401.2)</td>
</tr>
<tr>
<td>$G_{ss}$</td>
<td>Baseline glucose concentration</td>
<td></td>
<td>14§</td>
<td>9.8 (11.6)</td>
<td>10.3 (11.5)</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_I$</td>
<td>Volume of distribution</td>
<td></td>
<td>41*</td>
<td>31.8 (4.8)</td>
<td>31.8 (2.7)</td>
</tr>
<tr>
<td>$C_{LI}$</td>
<td>Insulin clearance</td>
<td></td>
<td>29*</td>
<td>26.9 (10.0)</td>
<td>26.9 (10.6)</td>
</tr>
<tr>
<td>$k_{IE}$</td>
<td>Effect delay rate constant, insulin on glucose clearance</td>
<td></td>
<td>58*</td>
<td>57.4 (7.0)</td>
<td>75.2 (39.9)</td>
</tr>
<tr>
<td>FPS, (mU)</td>
<td>First-phase insulin secretion</td>
<td></td>
<td>67§</td>
<td>127.7 (14.8)</td>
<td>110.9 (13.5)</td>
</tr>
<tr>
<td>$I_{ss}$</td>
<td>Baseline insulin concentration</td>
<td></td>
<td>49§</td>
<td>50.5 (13.1)</td>
<td>46.5 (11.1)</td>
</tr>
<tr>
<td>Parameter correlations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corr$_{VG-Q}$</td>
<td>Correlation between $V_G$ and $Q$</td>
<td></td>
<td>-0.19*</td>
<td>-0.732 (6.7)</td>
<td>-0.743 (8.8)</td>
</tr>
<tr>
<td>Corr$_{VG-VI}$</td>
<td>Correlation between $V_G$ and $V_I$</td>
<td></td>
<td>0.7225*</td>
<td>0.624 (5.6)</td>
<td>0.653 (4.2)</td>
</tr>
<tr>
<td>Corr$_{Q-VI}$</td>
<td>Correlation between $Q$ and $V_I$</td>
<td></td>
<td>-0.122*</td>
<td>-0.225 (19.3)</td>
<td>-0.313 (11.9)</td>
</tr>
<tr>
<td>Corr$<em>{CL</em>{GI}-FPS}$</td>
<td>Correlation between $CL_{GI}$ and FPS</td>
<td></td>
<td>-0.3364§</td>
<td>-0.389 (24.4)</td>
<td>-0.492 (20.5)</td>
</tr>
<tr>
<td>Corr$<em>{CL</em>{GI}-ISS}$</td>
<td>Correlation between $CL_{GI}$ and $I_{ss}$</td>
<td></td>
<td>-0.7181§</td>
<td>-0.737 (15.3)</td>
<td>-0.566 (17.3)</td>
</tr>
<tr>
<td>Corr$<em>{CL</em>{GI}-GSS}$</td>
<td>Correlation between $CL_{GI}$ and $G_{ss}$</td>
<td></td>
<td>-0.0948§</td>
<td>-0.103 (60.0)</td>
<td>0.160 (83.4)</td>
</tr>
<tr>
<td>Corr$_{FPS-ISS}$</td>
<td>Correlation between FPS and $I_{ss}$</td>
<td></td>
<td>0.2386§</td>
<td>0.214 (47.0)</td>
<td>0.338 (19.9)</td>
</tr>
<tr>
<td>Corr$_{FPS-GSS}$</td>
<td>Correlation between FPS and $G_{ss}$</td>
<td></td>
<td>-0.6058§</td>
<td>-0.622 (19.3)</td>
<td>-0.645 (14.0)</td>
</tr>
<tr>
<td>Corr$_{ISS-GSS}$</td>
<td>Correlation between $I_{ss}$ and $G_{ss}$</td>
<td></td>
<td>0.3957§</td>
<td>0.447 (22.8)</td>
<td>0.355 (25.0)</td>
</tr>
</tbody>
</table>

* Prior values
† Fixed to published value, $CL_G$ (diabetic), $K_{GE1}$ (healthy)
‡ Estimated with lower and upper boundary using healthy and diabetic values
§ Free estimation
|| RSE values (variance scale) were obtained from the bootstrap (n=100)
Visual predictive checks

*IVGTT-OGTT-IGI model among IGT subjects*

The VPCs for the IVGTT-OGTT-IGI model in this IGT subjects were shown in Figure 8. For both glucose and insulin components, the model fitted the median data of IVGTT and OGTT fairly well. An adequate model fit can be observed at the 2.5\textsuperscript{th} and 97.5\textsuperscript{th} percentile of the data for every component, especially for the insulin. A small model misspecification, however, was observed for the variability of the glucose component (at the 2.5\textsuperscript{th} and 97.5\textsuperscript{th} percentile), especially at the end of time profile. This might be explained by a large heterogeneity in term of glucose control in this populations, in which some of the subjects may be at the beginning of the IGT state close to the healthy state, whereas the other may be at the end, close to T2DM. Apart from the small misfit, this combination of IVGTT-OGTT-IGI model could adequately describe the data of IGT subjects.

*Figure 8.* VPCs of the IVGTT and OGTT for the glucose and insulin for the subject with IGT. Solid lines represent the median of observed data; dashed lines represent the 2.5\textsuperscript{th} percentile (lower part) and 97.5\textsuperscript{th} percentile (upper part) for observed data. Dark shaded areas are the 95\% confidence interval for median of simulated data, light shaded areas are the 95\% confidence interval for 2.5\textsuperscript{th} percentile (lower part) and 97.5\textsuperscript{th} percentile (upper part) from 500 simulated datasets.
**IVGTT-OGTT-IGI model with disease progression and intervention**

The VPCs for glucose and insulin components in control and intervention groups are shown in Figure 9 for IVGTT. The IGI model with the inclusion of natural disease progression and intervention effects has fairly well described the data for baseline and the fourth year for IVGTT. An adequate overall model fit can be seen for both glucose and insulin components in the baseline and the fourth year, among subjects in the control as well as lifestyle intervention groups. The median and the 5th percentile model prediction were very good for all cases. Only a small over-prediction can be found in the 95th percentile of the model prediction for the baseline glucose component. Possible explanation might be due to the heterogeneity factor. On the fourth year, the confidence interval for the model prediction was wider than at baseline, as the subjects who developed diabetes were excluded from the VPC analysis. The VPCs for OGTT are illustrated in Figure 10. A very good model fit can be observed on the median, 5th and 95th percentile of simulated data in all cases, except for the over-prediction of the 5th percentile of baseline glucose concentrations. The same possible explanation as for the misfit of baseline glucose for IVGTT may be applied in this case.
Figure 9. VPCs for IVGTT from baseline and the fourth year for final model of IVGTT-OGTT-IGI among IGT subject, with the diabetes disease progression and intervention effects. Solid lines represent the median of observed data; dashed lines represent the 5th percentile (lower part) and 95th percentile (upper part) for observed data. Dark shaded areas are the 95% confidence interval for median of simulated data, light shaded areas are the 95% confidence interval for 5th percentile (lower part) and 95th percentile (upper part) from 500 simulated datasets.
Glucose and insulin concentrations of fasting and 2-hours profiles

The overall model fit for the baseline and the 2-hour glucose and insulin was adequate, as illustrated in Figure 11. In specific, the model fit for the 2.5th percentile of the baseline glucose was a bit under-predicted in the control group. For the 2-hour glucose concentration, a misfit can be found at baseline (year 0), in which an under-prediction can be found. This misfit may be explained by a condition known as the regression toward the mean\textsuperscript{109}. The subjects were selected based on their 2-hour postprandial glucose in between 160-220 mg/dL, based on the IGT criteria form the WHO technical report series 1985\textsuperscript{95}, therefore, a tight variability for the baseline was observed with a specific mean value. However, for the subsequent observations (for example at the first year), the variability may increase by chance, in
which the subjects who had a value close to the mean at the first occasion, had a value that was further from the mean at the second one. For the insulin component, a small misfit can be found at the 97.5\textsuperscript{th} percentile and the later time point for the median data.

*Figure 11.* VPCs for baseline and 2-hour of plasma glucose and serum insulin concentration for final model of IVGTT-OGTT-IGI among IGT subject, with the diabetes disease progression and intervention effects. Solid lines represent the median of observed data; dashed lines represent the 2.5\textsuperscript{th} percentile (lower part) and 97.5\textsuperscript{th} percentile (upper part) for observed data. Dark shaded areas are the 95% confidence interval for median of simulated data, light shaded areas are the 95% confidence interval for 2.5\textsuperscript{th} percentile (lower part) and 97.5\textsuperscript{th} percentile (upper part) from 500 simulated datasets.
Sensitivity and specificity analyses

The performance of the model prediction was assessed with the sensitivity and specificity analyses. The results are summarized in Table 6, with the analyses calculated based on the doctors’ diagnosis as well as the observed data.

Doctors’ diagnosis

Based on the doctors’ diagnosis, the sensitivity analysis result was the best for scenario B (86%), which was by excluding the glucose and insulin concentrations for the IVGTT at the fourth year, followed by scenario A, by using all data (82%) and finally scenario C, by excluding the glucose and insulin concentrations at 0 and 120-minutes (68%). Even though scenario B had the best sensitivity, it had a lower specificity (87%) than scenario A (95%) but slightly higher than scenario C (84%).

Observed data

For the analyses results based on the observed data, a much lower sensitivity but a small difference in the specificity results as compared to the doctors’ diagnosis for all scenarios were observed. About 47% sensitivity recorded when using all data (scenario A) and excluding the glucose and insulin concentrations at the fourth year (scenario B), and 36% when excluding the glucose and insulin concentrations at 0 and 120-minutes (scenario C). However, for the specificity, higher values were observed in scenario A, B and C, which were 91%, 87% and 78%, respectively.

Table 6. The summary of the sensitivity and specificity analyses results based on the diabetes diagnosis made by the doctors’ (Doctors’ diagnosis) and by referring to the observed fasting and 2-hour glucose concentrations (Observed data), in 3 different scenarios: A, B, and C.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Doctors’</td>
<td>Observe</td>
</tr>
<tr>
<td></td>
<td>diagnosis</td>
<td>data</td>
</tr>
<tr>
<td>Scenario A</td>
<td>0.82</td>
<td>0.47</td>
</tr>
<tr>
<td>Scenario B</td>
<td>0.86</td>
<td>0.47</td>
</tr>
<tr>
<td>Scenario C</td>
<td>0.68</td>
<td>0.36</td>
</tr>
</tbody>
</table>
WHIG model (Paper (IV))

Natural disease progression and intervention effects model
The estimated parameters of the WHIG model in subjects with IGT are summarized in Table 7.

**Body weight**
For a typical individual in this IGT population, the estimated baseline body weight (BLWT) was 85.4 kg. The weight was estimated to decrease by 0.9%, and further decreased with the effect of intensive lifestyle intervention with individualized counselling by 3.2%, producing a net decreasing effect of 4.1%. The lifestyle intervention was more effective in lowering the weight than the standard counselling for the controls, as expected.

**Insulin sensitivity**
The mean estimated baseline insulin sensitivity in this population was 63.2% and 63.7% of normal for subjects in the control and intervention groups. A mean increase of 69.0% of normal in the control and 77.9% of normal in the intervention group was observed at the end of the study. A higher improvement of insulin sensitivity achieved with the intervention than without, as a result of weight changes.

**Beta cell function**
The baseline beta cell function in this population was estimated as a mean of 50.1% and 51.7% of normal for subjects in the control and intervention groups. At the end of the study, the mean beta cell function was decreased to 41.1% in the control and 40.1% of normal in the intervention group. A decrease beta cell function values for both groups in this model may be explained by the improvement of insulin sensitivity achieved from the weight changes. This may result in less need of insulin secretion, thus resulting in an apparent reduction in beta cell function from baseline.

**FSI**
Baseline FSI was estimated to be similar for the subjects on both control and intervention groups, as a mean value of 9.9 mU/L. A mean decrease of 0.9 mU/L (control) and 1.6 mU/L (intervention) was estimated at the end of study.

**FPG**
Baseline FPG was estimated to be as a mean value of 6.1 mmol/L for control and 6.0 mmol/L for intervention groups. A mean increase of 0.3 mmol/L (control) and 0.2 mmol/L (intervention) for FPG was estimated at the end of the study.
**HbA1c**

Baseline HbA1c was estimated to be similar for the subjects in the control and intervention groups, which was a mean of 5.8%. At the end of the study, a mean of 0.1% decrease in HbA1c was estimated for the subjects in the intervention group, whereas the mean of HbA1c for the subjects in the control group remained unchanged. In a typical individual, the total effect of both natural disease progression and lifestyle intervention has resulted in a 0.4% decrease in PPG input that influenced the rate production of HbA1c.

Table 7. Final parameter estimates with relative standard errors (RSE, %) and their respective inter-individual variability (IIV, %) of the WHIG model among subjects with impaired glucose tolerance with the logistic regression dropout model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value (RSE, %)</th>
<th>IIV (%) (RSE, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$, WGT, $d$</td>
<td>Half-life of weight component</td>
<td>96.9*</td>
</tr>
<tr>
<td>BLWT, kg</td>
<td>Baseline weight</td>
<td>85.4 (0.04)</td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_0$</td>
<td>Baseline insulin sensitivity, logistic function</td>
<td>-0.418 (8.1)</td>
</tr>
<tr>
<td>Scale EFs</td>
<td>Scaling factor for change in weight on insulin sensitivity</td>
<td>0.0514*</td>
</tr>
<tr>
<td><strong>Beta cell function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$B_0$</td>
<td>Baseline beta cell function, logistic function</td>
<td>-0.117 (6.8)</td>
</tr>
<tr>
<td>$R_B$, $y$</td>
<td>Rate of baseline beta cell function decrease per year, logits</td>
<td>0.0693 (0.7)</td>
</tr>
<tr>
<td><strong>HbA1c</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M_T$, $d$</td>
<td>Mean transit time of HbA1c</td>
<td>38.9*</td>
</tr>
<tr>
<td>$k_{in}$, HbA1c, %/d L/mmol</td>
<td>Rate constant, HbA1c compartments production</td>
<td>0.0129*</td>
</tr>
<tr>
<td>PPG, %/d</td>
<td>Residual HbA1c production rate independent of FPG</td>
<td>0.0653 (0.02)</td>
</tr>
<tr>
<td>Scale PPG,EFw</td>
<td>Scaling factor on PPG when time &gt;365 days</td>
<td>-0.00379 (6.9)</td>
</tr>
<tr>
<td><strong>Disease progression and treatment effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP$_W$, %</td>
<td>Effect of natural disease progression (DP) on weight input</td>
<td>0.908 (13.0)</td>
</tr>
<tr>
<td>INT$_W$, %</td>
<td>Effect of intervention (INT) on weight input</td>
<td>3.18 (13.1)</td>
</tr>
<tr>
<td>INT$_{RB}$</td>
<td>Effect of intervention (INT) on beta cell function</td>
<td>0.0191 (27.9)</td>
</tr>
<tr>
<td><strong>Residual errors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>Proportional residual error on weight</td>
<td>0.0344 (3.0)</td>
</tr>
<tr>
<td>FSI</td>
<td>Proportional residual error on fasting serum insulin</td>
<td>0.251 (0.3)</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Value</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>FPG</td>
<td>Proportional residual error on fasting plasma glucose</td>
<td>0.067 (0.9)</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Proportional residual error on glycated haemoglobin</td>
<td>0.049 (0.9)</td>
</tr>
<tr>
<td>M_FSI</td>
<td>Magnitude of FSI eps-eta</td>
<td>0.969 (0.1)</td>
</tr>
</tbody>
</table>

**Logistic regression dropout model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Parameter related to the baseline probability of dropout</td>
<td>-14.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>( \theta_{STime1} ) /year</td>
<td>Parameter related to the time effect</td>
<td>5.4 (0.6)</td>
<td></td>
</tr>
<tr>
<td>( \theta_{STime2} ) /year</td>
<td>Parameter related to the time effect after 4 years</td>
<td>0.842 (6.7)</td>
<td></td>
</tr>
<tr>
<td>( \theta_{Time} )</td>
<td>Power function for time effect (Hill factor)</td>
<td>0.197 (1.7)</td>
<td></td>
</tr>
<tr>
<td>( \theta_{DWGT} ) /kg</td>
<td>Parameter related to body weight change from baseline</td>
<td>0.0321 (13.1)</td>
<td></td>
</tr>
<tr>
<td>( \theta_{IS} )</td>
<td>Parameter related to for insulin sensitivity</td>
<td>-0.907 (2.6)</td>
<td></td>
</tr>
<tr>
<td>( \theta_{T2DM} )</td>
<td>Probability of dropping out when FPG ( \geq 7.8)mmol/L</td>
<td>0.42 (7.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Value fixed to the published model.†
† RSE values (variance scale) were obtained from bootstrap (n=500).
‡ The parameters with an additive IIV (B0, RB, IS0, DPW).

### The logistic regression dropout model

The final logistic regression dropout model is described in the equation 10a until 13c, describing the implementation of the dropout predictors, and the calculation of the probability of the dropout event in the logistic function (PROB) to occur and to not occur.

\[
P = \theta_{Base} + \theta_{STime1} \times \left( \frac{\text{Time}}{365} \right)^{ProTime} + \theta_{DWGT} \times (DWGT) + \theta_{IS} \times IS
\]

**Eq. 10a**

*Or if* \( \text{time} > 1460 \text{ days} \)

\[
P = \theta_{Base} + \theta_{STime1} \times \left( \frac{\text{Time}}{365} \right)^{ProTime} + \theta_{STime2} \times \left( \left( \frac{\text{Time}}{365} \right)^{ProTime} - \left( \frac{1460}{365} \right)^{ProTime} \right) + \theta_{DWGT} \times (DWGT) + \theta_{IS} \times IS
\]

**Eq. 10b**

\[
PROB = \exp(P) / (\exp(P + 1))
\]

**Eq. 11**

P in the equation 10a represents the probability of dropping out with linear predictors, in which the \( \theta_{Base} \) is the estimated parameter of the baseline predictor. Time is a predictor of dropout and it was investigated as a power function, with the probability of dropout increased with increasing time. The time effect on dropout was observed to be nonlinear, with estimated PTime
value of 0.3. The estimated parameter related to the time is described as $\theta_{STime1}$. The changes of weight from baseline (DWGT) and insulin sensitivity value were the most significant covariates in the dropout model and their estimated parameters are $\theta_{DWGT}$ and $\theta_{IS}$. The insulin sensitivity was calculated as the baseline insulin sensitivity ($ISS_0$) multiplied by the effect of weight change on the insulin sensitivity ($EFF_S$). In equation 10b, an additional slope for time function with the estimated parameter of $\theta_{STime2}$ was added to differentiate the time effect in the active study period with intervention (0-4 year) and the follow-up (4-6 year).

An improvement of the OFV by 12.9 was observed by adding the second slope of time ($STime2$), and further improvements by 5.1, 10.7 and 21.2 were obtained with the addition of the power function ($PTime$), change of body weight from baseline (DWGT) and insulin sensitivity (IS), respectively. Equation 11 represents the estimated probability of dropout in the logistic function (PROB), and it was constrained to lie between 0 and 1. The estimated parameters with their relative standard error are summarized in Table 7.

$$PD = 1 - (1 - PROB)^{DTime}$$  \hspace{1cm} Eq. 12a

*And if $FPG \geq 7.8\text{mmol/L}$*

$$PD = \theta_{T2DM}$$  \hspace{1cm} Eq. 12b

*And if $time < 365$ days*

$$PD = 0$$  \hspace{1cm} Eq. 12c

Equation 12a to 12c describe the probability of dropout (PROB), scaled to the time interval (DTime), which was 365 days. In addition, a separate probability of dropout was estimated when the $FPG \geq 7.8$ mmol/L, indicating the probability of dropout because of diabetes, as described in equation 12b. In equation 12c, a zero probability to dropout was also implemented when time < 365 days, indicating no dropout was allowed to occur at time 0.

$$PND = (1 - PROB)^{DTime}$$  \hspace{1cm} Eq. 13a

*And if $FPG \geq 7.8\text{mmol/L}$*

$$PND = 1 - \theta_{T2DM}$$  \hspace{1cm} Eq. 13b

*And if $time < 365$ days*

$$PND = 1$$  \hspace{1cm} Eq. 13c
The probability of dropout event to not occur was implemented as in the equation 13a to 13c, as a counterbalance from the probability of dropout event to occur.

All the parameter estimates for the logistic regression dropout model are summarized in Table 7. As expected, the baseline dropout was estimated to be -14.5, indicating a very low probability of dropout in the first interval (occasion = 0 to 1). A slightly lower probability of dropout in the active intervention than the follow-up period was observed. An increase in DWGT increased the probability of the dropout event to occur. In addition, the probability of dropping out was 42% when the subjects had an FPG ≥ 7.8 mmol/L, indicating less than 50% probability of subjects were diagnosed with diabetes based on the FPG.95

**Visual predictive checks**

The VPCs for the final model can be found in Figure 12 for each biomarker, and Figure 13 for their changes from baseline. The plots in the A section represent the final model with the inclusion of dropout model, whereas for the plots in B are without. An overall model fit was good for each biomarker, especially with the inclusion of dropout model. In specific, the inclusion of dropout model (A) was able to overcome the model over-prediction in the 95th percentile (B) of the FPG component. For the change from baseline, the same good model fit was illustrated in the VPCs, with or without the dropout model, except for the under-prediction at the 95th percentile of the HbA1c component, as well as a small over-prediction of the weight component for the first year, also at the 95th percentile.
Figure 12. VPCs of biomarkers measured in the WHIG model among IGT subjects, based on control and intervention group. The biomarkers are fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), weight and fasting serum insulin (FSI). A) VPCs for the final model with dropout model, and B) VPCs for the model without dropout model. Black circles indicate observed data. Solid lines represent the median of observed data; dotted lines represent the 5th percentile (lower part) and 95th percentile (upper part) of the observed data. Shaded areas are the 95% confidence interval for 5th percentile (upper part), median (middle part) and 95th percentile (upper part) from 500 simulated datasets.
Figure 13. VPCs of the change from baseline (cfb) of the biomarkers measured in the WHIG model among subjects with impaired glucose tolerance (IGT) subjects, based on control and intervention group. The biomarkers are fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), weight and fasting serum insulin (FSI). C) cfb VPCs for the final model with dropout model, and D) cfb VPCs for the model without dropout model. Black circles indicate observed data. Solid lines represent the median of observed data; dotted lines represent the 5th percentile (lower part) and 95th percentile (upper part) of the observed data. Shaded areas are the 95% confidence interval for 5th percentile (upper part), median (middle part) and 95th percentile (upper part) from 500 simulated datasets.
Figure 14 is the illustration of the Kaplan-Meier-VPCs (KM-VPCs) for the final dropout model (overall- and groups- stratified survival curves) by using only the categorical data. These KM-VPCs show a good model prediction on both overall survival and groups-stratified survival curves. The rate of dropout for the subjects in the intervention group was slower than the control for both the active part of the study and for the follow-up period. An adequate dropout model was successfully developed to describe the dropout event in the study, by using only categorical data, as well as with simultaneous analysis.

![Figure 14. Kaplan-Meier-VPCs for the logistic regression dropout model as an overall survival curve (upper panel) and as stratified on groups (lower panels), using only the categorical data. The solid blue line represents the observed data, and the shaded area is the 95% prediction intervals of the Kaplan Meier plot from 100 simulated datasets. The estimated parameters for baseline, STime1, STime2, PTime, and DWGT were -12.8, 3.36/year, 0.86/year, 0.27, and 0.031/kg.](image-url)
Discussion

Study design selection for the early phase clinical trial of anti-diabetes drug development

Inclusion vs. exclusion of insulin measurements

In this study, a smaller needed samples size has been translated to a higher study power with unchanged sample size. In most cases, the inclusion of insulin measurements added power to detect drug effects and to distinguish the correct drug MoA from incorrect ones. As expected, the increase in power with inclusion of insulin was strongest when MoAs were related to insulin (BINS and INCR), and weaker when the MoAs were related to the glucose (CLG, EGP, GABS). The insulin-dependent glucose clearance (CLGI) had little or no gain in power of including insulin when tested against the glucose related parameters. When discriminating CLGI from basal insulin secretion, either as competing MoAs or as a secondary MoA, insulin measurements have consistently contributed to a large improvement in power. A similar trend was also seen with CLGI and incretin. This behaviour is possibly related to this MoA being related both to glucose and insulin. Glucose sensitivity (GSEN), which also is related to both glucose and insulin, unlike CLGI, behaved much more as a pure insulin related parameters, with a large power increase with the inclusion of insulin measurements in the analysis.

The setting of this project was not feasible to distinguish a correct MoA on CLG from an incorrect MoA on EGP. There were more than 100 subjects were needed in a study, both with and without insulin measurements. This is not surprising as the insulin-independent glucose clearance and the endogenous glucose production (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 the tracer glucose in the design.

An adequate accuracy and a good precision for AUGC_{DPL} were obtained in all MoAs, with and without insulin measurements. This reflects a good model performance for the estimation of AUGC_{DPL}. The insulin measurements did not affect the precision of AUGC_{DPL}, reflected by the similar trends of accuracy and precision distribution when including and excluding insulin measurements.
Most appropriate study design based on the drug MoAs

The difference in number of observations between the designs could explained the results of the study power analysis. The designs differed greatly in number of observations: n=37, 26, 14 and 6 for MTT-24, IVGTT, GGI and OGTT/sMTT/NO, respectively. Thus, the IVGTT, GGI and MTT-24 have the highest study power due to the number of observations.

Besides than the number of observation, the study power is also dependent on the way glucose and insulin responses are provoked in the design. All drug effects, except on glucose absorption, affects the fasting concentrations of glucose and insulin. Thus, repeated fasting sampling at steady state or NO is expected to be powerful, with higher power the larger the change in fasting concentrations. Hypothetically, as insulin secretion increases, the relative importance of basal EGP, BINS and CLG will decrease. Thus, the power to detect drug effect on these MoAs will decrease the stronger insulin response the design provokes and the opposite will apply for CLGI.

The results showed that MTT-24 is the most powerful study design for drug MoA on basal insulin, due to the high number of observations. Furthermore, MTT-24 was not powerful for CLG while extremely powerful for CLGI because of the stimulation of high amount of insulin that makes CLGI more dominant. Moreover, the MTT-24 was more powerful to detect the drug MoA of decreasing GABS than sMTT because of the rich sampling, and more powerful than OGTT because of the slower glucose absorption rate that allows the detection of changes in absorption process.

GGI was the better study design for detecting CLG and EGP than IVGTT, but less powerful for CLGI. This may be explained by the fact that GGI stimulates insulin secretion in a step-up manner with longer period to reach high insulin levels compared to IVGTT. Additionally, IVGTT is the most powerful study design for CLGI, as it produces a rapid increase in glucose plasma level that stimulate high insulin secretion in an instant. The rich sampling of the intravenous glucose provocations may contribute to the high power to detect all drug MoAs, except for GABS.

In term of precision of parameter estimation, it has been shown that despite the biased parameter estimates in some of the models, a precise estimation of AUGC was observed reflecting a good model performance in term of simulation and estimation of the AUGC_D/PL,Cx.

MCMP method vs. ΔOFVgi/g in calculating study power

There are some methods used in pharmacometrics analysis to calculate the study power, such as the SSE\textsuperscript{103} and MCMP method\textsuperscript{80}. For SSE, it involves an intensive simulation and estimation methods, which is time-consuming and computer-intensive, thus making it less extensively used in the current time.
A newer method known as MCMP developed by Vong et al., is a faster and lesser computer-invasive alternative of calculating study power than the gold standard SSE\textsuperscript{76,80}. The MCMP method was initially used in Paper I for the study power calculation, however, the study power obtained was very high, with 3 included subjects giving 90% power at $\alpha=5\%$ in most MoAs. Furthermore, in some cases, less than 3 subjects were needed to achieve 95% study power at $\alpha=5\%$, especially when insulin measurements were included in the analysis.

The high power is related to the method of analysis (model-based), the size of the drug effect, and the design (cross-over, no. of observations and no. of subjects). Changing the method of analysis was not an option as the purpose was to investigate the performance of model-based analysis. The size of the drug effect had been selected based on what was a reasonable response for a new compound in development and a smaller drug effect would not be taken forward in the development.

The design in anti-hyperglycaemic drug development is fairly standard, with cross-over in early phase studies with observations every 30 minutes. This left the number of subjects as the comparable entity and an alternative approach of calculating relative power was suggested; the ratio of $\Delta OFV_{\text{full-reduce}}$ for analysis with insulin and the $\Delta OFV_{\text{full-reduce}}$ for analysis without insulin, which reflects the fraction of subjects needed to achieve equal study power with and without insulin.

To evaluate the reliability of suggested approach, the example of results from the MCMP method was also compared to the $\Delta OFV_{\text{gi/g}}$ method for Part 1 of Paper I (refer to Table 1), in which similar results obtained for the ratio of number of subject needed, for both methods. This reflected the ability of $\Delta OFV$ ratio power calculation to produce the results with an adequate similarity to the MCMP, even without a specific predetermined study power.

**Recommendation on study design selection based on drug MoAs and purpose of analysis**

Based on the results from Paper I and II, an overall written summary was developed to aid designing the studies of anti-diabetes drug development using model-based analysis. This guidance can be used in a scenario when the IGI model is used, involving a certain drug MoAs. The best study design with the inclusion or exclusion of insulin measurements for different analysis purposes and its less invasive alternatives for each hypothetical drug MoA was summarized in Table 8.
Table 8. Recommendation on design in relation to purpose of the analysis and hypothesized site of drug actions.

<table>
<thead>
<tr>
<th>Purpose of analysis</th>
<th>MoA</th>
<th>Study power and AUGC&lt;sub&gt;IO/PL&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Drug parameter estimates&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Identification of drug action</th>
<th>Identification of correct site of drug action</th>
<th>Identification of secondary true site of drug action</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Insulin reduced sample size for all MoAs</td>
<td>Insulin reduced sample size for all but competition with CLG, EGP, GABS</td>
<td>Insulin reduced sample size for all</td>
</tr>
<tr>
<td></td>
<td>CLGI</td>
<td>IVGTT (OGTT is the less invasive alternative)</td>
<td>IVGTT (OGTT is the less invasive alternative)</td>
<td>Insulin reduced sample size for all MoAs</td>
<td>Insulin reduced sample size for all but competition with CLG, EGP, GABS</td>
<td>Insulin reduced sample size for all</td>
</tr>
<tr>
<td></td>
<td>EGP</td>
<td>GGI (NO is the less invasive alternative)</td>
<td>GGI (NO is the less invasive alternative)</td>
<td>Insulin reduced sample size for all but competition with CLG</td>
<td>Insulin reduced sample size for all, but competition with primary CLG and CLGI</td>
<td>Insulin reduced sample size for all</td>
</tr>
<tr>
<td></td>
<td>CLG</td>
<td>GGI (sMTT is the less invasive alternative)</td>
<td>MTT-24 (sMTT is the less invasive alternative)</td>
<td>Indiscriminable from EGP</td>
<td></td>
<td>Indiscriminable with primary EGP</td>
</tr>
<tr>
<td></td>
<td>GABS</td>
<td>MTT-24 (sMTT is the less invasive alternative)</td>
<td>MTT-24 (sMTT is the less invasive alternative)</td>
<td>Insulin reduced sample size for all</td>
<td>Insulin reduced sample size for all</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BINS</td>
<td>MTT-24 (NO is the less invasive alternative)</td>
<td>IVGTT (NO is the less invasive alternative)</td>
<td>Insulin reduced sample size for all</td>
<td>Insulin reduced sample size for all</td>
<td></td>
</tr>
<tr>
<td></td>
<td>INCR</td>
<td></td>
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<td>Insulin reduced sample size for all</td>
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<td></td>
<td>GSEN</td>
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<td>Insulin reduced sample size for all</td>
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</tbody>
</table>

<sup>1</sup>Insulin did not improve accuracy or precision  
<sup>2</sup>Not tested in Paper II  
<sup>3</sup>Not tested in Paper I (Part 3)

Abbreviation: SSE = stochastic simulation and estimation, gi = glucose and insulin, g = glucose only, INCR = incretin activity, BINS = basal insulin secretion, CLG = insulin-independent glucose clearance, CLGI = insulin-dependent glucose clearance, EGP = endogenous glucose production, GABS = glucose absorption, GSEN = glucose sensitivity.

IVGTT-OGTT-IGI model in IGT population

The IGI model was published with the final parameter estimates for OGTT and IVGTT among diabetes subjects and healthy volunteers. The use of NONMEM’s prior functionality to develop the IGI model among IGT population was considered as the best, as the functionality incorporates the pub-
lished parameter values with their uncertainty taken into consideration during estimation. Therefore, the final parameter estimates in the new population (IGT) are comparable to the published (healthy and T2DM). Besides, a more stable population data analysis has been produced by this approach, especially in the case of a complex pharmacokinetics/pharmacodynamics model as the IGI.99

The CL$_{G}$ was observed to reach the lower boundary during estimation, most probably due to its non-identifiability resulting from the absence of hot glucose data in this IGT population. The hot glucose was used with the glucose and insulin data during the first development of IGI model,55–57 to differentiate between the exo- and endogenous glucose sources and to help discriminate the CL$_{G}$ from the CL$_{GI}$. A similar observation seen for the k$_{GE1}$, as it was estimated to be unreasonably high, probably as a compensation of the nearly absence of GPRG value in this IGT population.

On the population level, the CL$_{GI}$, FPS and GPRG that translate to insulin sensitivity, insulin secretion function as a result of high glucose load, and feedback mechanism function of glucose on its own production were close to the state of diabetes mellitus. The BIO$_{G}$ however, was close to the healthy state.

Diabetes disease progression and lifestyle intervention effects in the IGT population

Insulin sensitivity

In this thesis, the change of insulin sensitivity was assessed as the change of the parameter CL$_{GI}$ in Paper III, and directly as insulin sensitivity estimation in Paper IV. In Paper III, a decrease in CL$_{GI}$ over time due to the natural disease progression indicating the depletion of the insulin sensitivity over the years. Similar result found in Paper IV, as the insulin sensitivity in this IGT population was about 63% of normal for the baseline, reflected as the deterioration of insulin sensitivity compared to healthy people. This was in agreement with other studies’ results that reported a reduction in the insulin sensitivity translated from 20-30% reduction of the insulin-dependent glucose clearance among IGT, from the NGT subjects,10,15,110–112 even after BMI adjustment.110 The combination of the insulin secretion depletion and muscle insulin resistance increase were determined to be the most prominent causes of decreasing insulin-dependent glucose clearance among the IGT subjects.113

With lifestyle intervention, a slower deterioration of insulin sensitivity was observed in Paper III, translated from the final net effect of a smaller reduction of the CL$_{GI}$ over time. In Paper IV, as a result of weight reduction, the insulin sensitivity was significantly increased at the end of the study,
with a higher increment in the intervention group than the control. These results complement the results from previous studies among the IGT subjects, regarding the benefit of lifestyle interventions (counselling on dietary intake, weight reduction, and moderate intensity physical activities for at least 150 minutes per week) on insulin sensitivity and insulin secretion as a result of improving beta cell function.\textsuperscript{26–28} A study reported the improvements of glucose tolerance and insulin sensitivity in the lifestyle intervention group subjects, determined by weight loss and increased physical fitness.\textsuperscript{26} Another study concluded that the lifestyle intervention significantly reduced the diabetes incidence among IGT subjects, due to the improvement of beta cell function and insulin sensitivity.\textsuperscript{27} In addition, two studies reported a 58% reduction of the progression from IGT to T2DM throughout the mean follow-up duration of 2.6 to 3.2 years with lifestyle intervention.\textsuperscript{24,25}

**Beta cell function**

In Paper III, the beta cell function was assessed from the change of FPS parameter. It was found to decrease every year, representing the deterioration of acute beta cell function response to secrete insulin following a high glucose concentration after the IVGTT. In Paper IV, the baseline beta cell function was about half of normal and reduced to a significant extent as a result of natural disease progression. The current results were supported by a number of studies reported the suboptimal beta cell function among IGT subjects in the form of lesser first- and second-phase insulin secretions, as compared to the NGT subjects.\textsuperscript{10,15,26,27,110,111} Other study concluded a higher reduction of the first-phase insulin secretion among IGT subject with the first degree relative with T2DM than those without.\textsuperscript{114}

The acute insulin secretion from pancreatic beta cell had a much slower deterioration with lifestyle intervention, marked by a very low decrease of the estimation on the FPS following the IVGTT over the years. Previous publications have recorded the improvement of beta cell function among IGT subjects, with the effect of lifestyle intervention.\textsuperscript{26–28} However, in Paper IV, the beta cell function was estimated to decrease more in the intervention group than the control. These contradictory results can possibly be explained by the fact that the beta cell reduced its insulin production due to increased insulin sensitivity in the glucose-insulin regulation system, in which not equally much insulin secretion is needed to maintain the blood glucose, and this may be seen as a reduction of beta cell function. A paper by Bergman \textit{et.al} has illustrated this condition in which the improvement of insulin action (decreased insulin resistance) has resulted in a downregulation of beta cell sensitivity to secrete insulin.\textsuperscript{115} In addition, in the WHIG model (Paper IV), the old implementation of the HOMA equation is used to calculate the beta cell function, which has been shown to produce a slightly lower beta cell function than expected.
Weight
The weight change was investigated in Paper IV, and it was shown to decrease more effectively for the subjects in the lifestyle intervention group than control. The weight had slightly reduced without intervention, possibly due to the placebo effect, as the subjects in the control group were also provided with brief oral and written information about diet and exercise recommendation at baseline and subsequent visits. This might encouraged the subjects to lead a healthier lifestyle, for example changing the dietary and physical activities, which might reduce the overall weight. The subjects in the lifestyle intervention group, however, were provided with the individualized detailed advice to achieve the goals of intervention regarding diet and exercise recommendations. They were observed to benefit directly from the intervention, as reflected by a greater extent of weight reduction as compared to the subjects in the control group. Previous studies have shown the benefit of lifestyle intervention, for example with counselling on dietary intake and moderate intensity physical activities for at least 150 minutes per week, that effectively reduced weight among IGT subjects. A similar results on the weight reduction obtained from a study involving the NGT, IGT and T2DM subjects.

FPG
The FPG was estimated to be around a mean of 6.0 mmol/L at baseline, which can be categorized into the prediabetes state. As explained earlier, the FPG value was not as high as in T2DM patients, probably due to increasing baseline insulin secretion to prevent the increment of glucose level among IGT subjects. The FPG was almost unchanged through the end of the study, which is expected as subjects who developed diabetes were excluded and only those with unchanged or even decreased FPG remained in the study. Thus, only those subjects who had improved insulin sensitivity or insulin secretion and managed to maintain normoglycaemic levels stayed in the study.

FSI
The change of FSI was investigated as the change in $I_{ss}$ in Paper III, and a direct FSI estimation in Paper IV. The $I_{ss}$ was found to increase from the baseline to the first year, and the estimated FSI value (mean = 9.9 mU/L) suggesting an increase in the beta cell function burden to secrete higher insulin secretion than normal. This may be explained by the feedback mechanism of beta cell to secrete more insulin as a result of the decreasing $CL_{Gi}$ (insulin sensitivity) and increasing insulin resistance, especially in the early progression of T2DM. In this condition known as compensatory hyperinsu-
linemia, a higher insulin secretion is needed to suppress the increase of baseline glucose concentration due to increased insulin resistance. This feedback mechanism phenomenon has been well documented. Besides, it was documented in previous studies that fasting plasma insulin concentration was higher in the IGT than in NGT subjects. Another study reported a higher baseline insulin secretion rate among the IGT than the normal glucose tolerance counterparts.

Lifestyle intervention affected the $I_{ss}$ value by an increment from baseline until the first year, and later maintained throughout the remaining years. The estimated FSI in Paper IV, however, was decreased at the end of six years in this study. For both of these conditions, the possible explanation might be related to the physiological improvement of beta cell to secrete insulin at the baseline level, with the effect of lifestyle intervention. Furthermore, it may also be related to the improvement of insulin sensitivity, as the indirect effects of the lifestyle intervention and weight reduction. Previous studies reporting the reduction of FSI with the effect of lifestyle intervention, which related to the improvement of insulin sensitivity.

**HbA1c**

In Paper IV, the baseline HbA1c was estimated to be a mean of 5.8%, denoted a prediabetes state, and slightly improved by a 0.1% decrease at the end of the study. This slight improvement may be related to the unchanged glucose value throughout the six years of study that influence the production of a stable HbA1c value. Previous studies have documented a decreased HbA1c value among prediabetes subjects and T2DM patients who received lifestyle intervention, due to the improvement of glucose control. Besides than the FPG, the PPG input was also contributed to the HbA1c production. In this study, the effect of lifestyle intervention resulted in a decrease in PPG that drove the rate production of HbA1c. A few studies have documented the improvement of meal intake, reduced energy intake, decreased consumption of and quantity of fat, increased vegetables intake, as well as decreased sugar, salt and alcohol among subjects who underwent lifestyle intervention programme.

**Specificity and sensitivity analyses**

In the Paper III, the sensitivity and specificity analyses were performed to assess the model prediction performance, on 3 difference scenarios:

A. using all data from 0- until 120-minutes for the OGTT and IVGTT from baseline to the fourth year, as a reflection of best-case scenario.

B. excluding the glucose and insulin concentrations of the IVGTT at the fourth year, to eliminate the influence of the rich IVGTT data on the
natural disease progression and intervention effects, and on the model predictability of subjects who will developed diabetes.

C. excluding glucose and insulin concentrations at the 0- and 120-minutes, to let the model predict the missing observations based on the estimation of the natural disease progression and intervention effects, as the baseline and 2-hour glucose are used for the diagnosis of T2DM. Thus, the predictions of the values for diagnosis were purely model-driven.

Based on the doctors’ diagnosis, an acceptable sensitivity and specificity analyses results were obtained. Specifically, the final model could identify 68% to 86% subjects who developed T2DM and only 14% to 32% went undetected. The model was also able to identify 84% to 95% subjects who did not develop T2DM, with only 16% to 5% falsely identified to develop T2DM at the fourth year. These results were also suggesting that the glucose and insulin concentrations at the fourth year may have an influence on the specificity, but not sensitivity analysis, by referring to the 86% sensitivity and 87% specificity for the scenario B, as compared to 82% sensitivity and 95% specificity for the scenario A. Besides, the model could predict an acceptable 0- and 120-minutes glucose and insulin concentrations from the baseline until the fourth year, reflecting from a good sensitivity (68%) and specificity (84%) of the model.

Based on the data, however, a poor sensitivity with less than 50% was obtained for all scenarios. The specificity, on the other hand, was good, with 78% to 91%, indicating a high model performance to correctly identify the person who were never progress into T2DM, but quite poor to identify the subjects who developed T2DM at the fourth year. From the diabetes diagnosis record, it was observed that more subjects were supposed to developed diabetes if the diagnosis was made directly on the observed data (FPG and 2-hour glucose), than from the diabetes diagnosis done by the doctors. This may be related to the fact that a second OGTT was performed to confirm the T2DM diagnosis if any subjects had a high FPG or the 2-hour glucose level on the first reading. Unfortunately, the data of the second OGTT were not available. Most probably in most cases, the second OGTT had ruled-out the diabetes diagnosis, therefore less subjects were diagnosed as T2DM, as compared to when the diagnosis were made directly from the observed data.

Logistic regression dropout model

In the dropout model, the probability of dropout event to occur was higher as the time progress, but lower in the active study than the follow-up period. This could be explained by the possibility that the subjects were more motivated to stay in the study during the intervention period than after the end of the intervention. In addition, an increase in body weight from baseline has
shown to increase the probability of dropping out. This may be related to a higher probability to progress into T2DM for the subjects who had increased in weight, thus leads to diabetes dropout. A decrease in motivation to stay in the study when failing to reduce weight could also be one of the factors. Besides, in the simultaneous analysis, the higher the insulin sensitivity, the lower probability of dropping out. The improvement of insulin sensitivity may be reflected by a better glucose control, especially for the 2-hour glucose level. A better glucose control leads to a lower progression to T2DM, thus reduced the probability of dropping out due to T2DM. For the dropout event related to the value of FPG, it was independent on the time component (constant), and 42% of the subjects who had FPG ≥7.8 mmol/L had dropped out because of T2DM. This low probability of being diagnosed with T2DM based only on FPG is also seen in the observed data, where there are a fair number of subjects with FPG ≥ 7.8 mmol/L who have remained in the study.
Perspectives

Currently, the pharmacometrics analysis in the anti-diabetes drug development has become more common, and may be considered as the standard approach. The studies related to the study design in the early clinical trial in this thesis have provided an insight on the most appropriate study design depending on the MoAs of the drug and the purpose of analysis, specifically when using the model-based analysis and the IGI model. A written guidance was produced for the future references, with the hope to maximise the benefit of using pharmacometrics analysis in the process of developing anti-diabetes drugs. An alternative way of calculating study power has also been used in this thesis, which has shown to be comparable to the standard approach of calculating study power. The future investigations may include mixed study designs, for example a study design of repeated fasting samples followed by intravenous glucose provocations such as the GGI or IVGTT. The mixed study design is expected to be informative on both dynamic and steady state effects, however, if the drug is expected to produce one of the studied MoAs as in the thesis work, intravenous provocations may be sufficient. Furthermore, the work in the thesis has also describes a methodology to investigate more combinations of drug MoAs in the future. The combination of two drugs with different MoAs could also be investigated. In this case, as the secondary drug effects has been well detected and differentiated from the primary ones in this thesis, we could expect that the separate effects of two combined agents could also be well, or might be even better, characterised. This is because the differences in pharmacokinetics between two drugs would improve the power to separate drug effects.

The quantification of the natural disease progression and lifestyle intervention effects in this thesis has helped us understand the physiological changes of the glucose and insulin regulation in the IGT population. In this thesis, the assessment of multiple short-term glucose provocations (IVGTT and OGGT) was combined to predict the long-term disease progression, and offers apart from the assessment of the onset of T2DM, also the framework for how to perform similar analysis, combining short-term data to predict long-term outcome. The developed IGI model in the IGT population may be useful for clinical trial simulations related to the IGT population. With the additional disease progression model and the quantification of the effect of lifestyle intervention, the model had also been used to quantify the long-term effects of non-medication lifestyle intervention on the changes on the glu-
cose and insulin regulations among the IGT subjects in this thesis. Further investigations on the effects of medication interventions can be conducted in the future. A longer longitudinal data, for example a 10 years’ study period and a more frequent sampling of OGTT, for example every 30 minutes from 0 until 240 minutes could benefit the analysis in better quantifying the disease progression and intervention effects. Moreover, the thesis work has also helped us to understand the effect of weight changes on the glucose and insulin homeostasis, specifically on the insulin sensitivity, beta cell function and HbA1c among IGT population, from the pharmacometrics point of view. Different approaches on analysing insulin sensitivity and beta cell function had also been performed in this thesis using different pharmacometrics models.

The implementation of the logistic dropout model in this thesis provides the understanding of the complex relationship between dropouts from study and weight as well as glucose changes. In the investigations, the most significant predictors of overall dropout have been identified. Future investigations may include the time-to-event modelling with the use of parametric models to describe the absolute risk of developing T2DM and the relationship to weight change, and changes in glucose covariates. This model could then be coupled with the model developed in the thesis and then used to simulate the risk of diabetes for various scenarios in terms of weight reduction.
Conclusion

This thesis has provided a first written guidance on how to design a study for pharmacometrics analysis when characterising drug effects for further improving the early phase of anti-diabetes drug development, and has characterised and quantify the progression from prediabetes to overt diabetes, both the natural progression and the progression with diet and exercise interventions, using pharmacometrics modelling.

In specific:

- The need of insulin measurements in designing a clinical trial of anti-diabetes drug using the pharmacometrics analysis and the IGI model was assessed using the study power analysis. Excluding the insulin measurements from analysis using the IGI model will affect the power to detect a drug effect, distinguish a correct drug MoA from an incorrect and detect a secondary MoA from a primary. However, insulin measurements will not impact the estimates of drug effect parameters.

- The most appropriate study design in phase I for the anti-diabetes drug development for several hypothetical MoAs was different for each MoA, depending on if the focus is the power the identify drug effect or accuracy and precision of drug effect parameter.

- A natural diabetes disease progression model was successfully added in the combination of IVGTT-OGTT-IGI model to describe parameter changes of FPS, CL_{GI} and Iss among the IGT subjects, with the quantification of the effects of lifestyle intervention.

- The effects of natural disease progression and lifestyle intervention on the body weight, insulin sensitivity, and beta cell function among IGT subjects were quantified in the WHIG model, with the addition of those effects on the PPG input. The model also includes the complex relationship between dropout from study as well as weight and glucose changes.
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